Volume 6
Number 82 *Transmissibility and pathological effects of the Mosaic Disease*
Article 1

February 1925

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February, 1925 Research Bulletin No. 82

Transmissibility and Pathological Effects of the Mosaic Disease

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AGRICULTURAL EXPERIMENT STATION
IOWA STATE COLLEGE OF AGRICULTURE
AND MECHANIC ARTS

BOTANY AND PLANT PATHOLOGY SECTION

AMES, IOWA

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TRANSMISSIBILITY AND PATHOLOGICAL EFFECTS OF THE MOSAIC DISEASE

By O. H. ELMER*

The investigations reported in this publication deal chiefly with the question of the transmissibility of mosaic disease, both artificially and thru the use of certain insect vectors. Another portion of the report deals with studies which have been made concerning the pathological effect of mosaic in plants and its relation to environmental conditions.

Mayer's (46) discovery in 1885 that mosaic of tobacco *(Nicotiana tabacum)* is a transmissible disease, coupled with Iwanowski's (34) demonstration that the filtered juice from diseased plants retained its infectivity, opened a new field in plant pathology. At the present writing mosaic is only one of many transmissible virus diseases of plants and animals. Altho the number of virus diseases of plants has been materially augmented during the last 44 years, our knowledge of their biological aspects has grown less rapidly. Mosaic has been found and described on many species of widely different families and orders, but there is little experimental evidence as to its host specificity.

Westerdijk (62), Allard (3), Jagger (37), and Schultz and Folsom (55) concluded from their experiments that certain mosaic diseases are restricted in their host range. Evidence, on the other hand, has been obtained showing that mosaic is not specific as to species, genera, families, or orders.

MOSAIC TRANSMISSION

Altho the infectious nature of the mosaic disease within the species *Nicotiana tabacum* was established by Mayer in 1885, this disease was not shown to be transmissible from one species to another until 1907 when Clinton's (18) investigations proved that it could be cross inoculated from tomato to tobacco and vice versa. Previously Koning (42) was unsuccesful in securing mosaic infection from tobacco to various species of the Solanaceae that are now known to be susceptible to this disease. Westerdijk was unable to communicate mosaic from tomato to tobacco. Iwanowski stated that *Datura stramonium, Hyoscyamus niger* and *Nicotiana rustica* were immune to the mosaic disease.

This paper was presented to the Graduate Faculty of Iowa State College in partial fulfillment of the requirements for the degree Doctor of Philosophy.

The writer wishes to express his appreciation for the helpful advice and criticism given during the course of these investigations by Dr. I. E. Melhus, under whose direction this work was done.

MOSAIC CROSS INOCULATIONS WITH SPECIES BELONGING TO THE SAME FAMILY.

The first extensive successful cross inoculations with the mosaic disease were reported in a preliminary article by Allard (1) in 1912. By artificial inoculations Allard (2) transmitted this disease to *Nicotiana tabacum, Lycopersicon esculentum, Petunia violacea, Physalis sp. Datura stramonium, Hyoscyamus niger* and *Capsicum sp.* At this time Allard reported failure to secure infection in *Solanum tuberosum, Nicotiana glauca, N. glutinosa* (not *N. viscosum*), *Solanum melongena* and *Atropa belladona*, but in later attempts he (4) succeeded in securing infection to *Nicotiana glauca* and *N. glutinosa*. Melhus (49) in 1922 first reported artificial mosaic infection of *Solanum melongena*. A large number of species of the Solanaceae, included in nine genera, are now known to be susceptible to the virus of tobacco mosaic.

Transmissibility of the mosaic disease in the Cucurbitaceae was first demonstrated by Doolittle (23) and by Jagger (35) whose papers appeared simultaneously. Doolittle and Walker (27) state that eight genera, including 23 species of the Cucurbitaceae, are susceptible to mosaic.

The mosaic disease of the Leguminosae has been successfully transmitted to species of *Soja, Trifolium, Melilotus, Lathyrus, Vicia, Phaseolus, Medicago, Pisum* and *Vigna*. Taubenhaus (57) in 1914 secured infection from mosaic to healthy sweet peas *(Lathyrus odoratus)* both artificially and by means of aphids. Stewart and Reddick (56) reported success in artificially transmitting mosaic from infected to healthy bean *(Phaseolus vulgaris)*. McLarty (47) reported successful artificial infection from mosaic to healthy sweet clover *(Melilotus sp.)*. Gardner and Kendrick (30) were successful in artificially transmitting mosaic from infected to healthy soybeans *(Soja max)*, while Dixon (19) secured mosaic cross infection among different species and genera of the Leguminosae.

Brandes (8) in 1919 showed that the mosaic disease of sugar cane *(Saccharum officinarum)* was transmissible. He utilized aphids to inoculate *Sorghum sp., Panicum sp., Syntherisma sanguinalis* and *Chaetochloa lutescens* from sugar cane. The following year Brandes (9) reported successful infection from sorghum to corn thru the medium of aphids and in a later publication (10) he reported artificial infection from diseased to healthy sugar cane. Chardon and Veve (14) secured mosaic cross infection thru the medium of aphids from sugar cane to *Syntherisma sanguinalis, Eleusine indica,* and *Echinochloa colona.*

Many species of plants belonging to additional families are susceptible to mosaic disease. Some of the species in which the

infectious nature of the disease has been demonstrated experimentally are: *Beta vulgaris* by Townsend (58); *Amaranthus retroflexus* by Doolittle (27); *Phytolacca decandra* by Allard (5); *Brassica sp.* by Schultz (54); *Rubus sp.* by Rankin and Hockey (53); *Apium graveolens* by Poole (52); *Martynia louisiana* by Doolittle (24); and *Lactuca sativa* by Jagger (39).

MOSAIC CROSS INOCULATIONS WITH SPECIES BELONGING TO DIFFERENT FAMILIES

Mosaic cross infection, in addition to occuring among species and genera of the same family, has been reported among species belonging to different families and orders. Jagger (37) secured artificial mosaic cross infection from cucumber to *Helianthus debilis* and to *Lobelia erinus* var. *gracilis*. Doolittle (25a) secured infection from mosaic cucumber to *Asclepias syriaca, Capsicum annuum* and *Martynia louisiana*, while Doolittle and Walker (26) reported results indicating that aphids from mosaic cucumber plants transmitted virus to *Solanum tuberosum* and *Phytolacca decandra*, and from mosaic potato back to cucumber.

Doolittle and Walker (27) later reported successful cross infection of mosaic from *Asclepias syriaca* to *Martynia louisiana* and to *Capsicum annuum* and secured infection from inoculations with the mosaic virus from cucumber to *Amaranthus retroflexus* and to *Physalis sp.*

In an earlier paper the writer (28) reported successful cross infection of mosaic from *Cucurbita pepo* var. *condensa* to *Petunia violacea, Nicotiana tabacum* and *Lycopersicon esculentum;* from *Nicotiana tabacum* and *Lycopersicon esculentum* to *Cucurbita pepo* var. *condensa;* from *Cucumis sativus* to *Nicotiana tabacum;* from *Nepeta cataria* to *Lycopersicon esculentum;* and from *Cucurbita pepo* var. *condensa, Solanum melongena*, and *Solanum tuberosum* to *Vigna sinensis*. Before presenting the data concerning the mosaic cross inoculation experiments it may be well to describe the materials and methods that were used in these investigations.

MATERIALS AND METHODS

In order to secure further information concerning the transmissibility of mosaic, considerable study was devoted to methods of inoculation. A description of the materials used and the methods of inoculation follow:

MATERIALS USED.

Vigorously growing young plants were used in testing the transmissibility of the mosaic virus. It was learned early that such plants are more suitable to mosaic inoculation experiments than slow growing or mature plants. The plants were grown in

pots where the period of vigorous growth is comparatively short and it was necessary to inoculate them when young. Tobacco plants were allowed to reach the rosette stage and the inoculations were made previous to the development of a central stalk. Tomatoes were inoculated when from 6 to 12 inches tall and cucumbers were used for the inoculations after the plants had produced from four to eight leaves. Beans, soybeans, and cowpeas were inoculated soon after the first true leaves were well developed.

Infections are more easily obtained when the inoculum is from vigorously growing host tissue. The mosaic infected tissue used for inoculations has consequently been obtained from near the growing tips of infected plants including juvenile leaves and in many cases including meristematic portions of the stems.

Owing to the infectious nature of the mosaic virus, all possible precautions are necessary to avoid accidental infection of plants. Two greenhouses were used for the investigations, one for the propagation of healthy mosaic-free plants where inoculated plants were kept between the time of inoculation and the time mosaic symptoms were first manifested. Inoculated plants and checks were kept side by side on the bench during the mosaic incubation period.

Additional precautions were taken to guard against accidental infection during the incubation period by using four insect proof cages, built by covering all six sides of suitable frames with a good grade of muslin; a portion of one side was provided with an opening that could be securely closed. The cages were placed on a rack three feet above the bench at one end of the mosaic free greenhouse. Inoculated plants with the checks were placed in the cages immediately after the inoculations and the plants kept therein during the entire incubation period.

Immediately following the appearance of visible symptoms of mosaic on inoculated plants, such plants were removed to a greenhouse not adjoining the mosaic free house. The greenhouse immediately adjoining the mosaic free house was used for the propagation of grasses and at no time were mosaic infected plants noticed in this greenhouse.

For the control of aphids, the mosaic free house was regularly fumigated with nico-fume paper or powder at intervals of one week during the fall, winter and early spring months; during the spring and summer, fumigations were made twice each week. At no time during the experiments were aphids found in the mosaic free greenhouse. Mealy bugs (*Pseudococcus maritimus* Ehr.*) earlier found by the writer (28) to transmit the mosaic disease, were kept under control by discarding any

*Identified by Harold Morrison, Bureau of Entomology, U.S.D.A.

plant found infested. White fly (*Aleyrodes vaporariorum* Westw.) was not found to transmit the mosaic disease, which confirms the results of Allard (4) and Doolittle (24). Nevertheless the greenhouse was fumigated at intervals with hydrocyanic acid gas to prevent the development of white fly infestations.

Care was taken thruout these investigations to avoid accidental contamination of the plants used. Previous to making inoculations, no mosaic infected plants were touched except those from which the inoculum was taken. In most cases the inoculations were made in the morning or immediately after entering the greenhouse, before other plants were touched. Just before making the inoculations, the hands were thoroly washed even tho no mosaic plants had been handled previously.

METHODS OF INTRODUCING INOCULUM.

Different methods of making inoculations were used in these investigations, including: (1) hypodermic needle injections of filtered juice from mosaic plants; (2) injections of juice under long continued pressure produced by a mercury column in a manometer; (3) insertion of fragments of mosaic infected tissue into healthy plants; (4) punctures thru drops of juice from infected plants; and (5) insect vectors.

Hypodermic Needle.

The hypodermic needle method was used in certain of the earlier experiments. By this method filtered juice from mosaic infected plants was injected near the growing point into the plant to be inoculated. Sterilization of all apparatus used in this method was effected by boiling.

Manometric Pressure.

This method was used where the injections were to be made under long continued pressure. A glass tube, one end of which was drawn to a capillary point, served as an inoculating tube. This tube was filled with filtered juice from mosaic infected plants and the finely drawn out end was inserted into the plant to be inoculated. The large end was connected with a short rubber tube to a glass manometer and sufficient mercury was poured into the open end of the manometer to exert a pressure on the juice, slowly forcing it into the plant. A column of mercury at an initial height of about seven centimeters was used to produce this pressure. In order to prevent the escape of the juice at the point where the inoculating tube entered the plant, the union was sealed with melted paraffin before the mercury was poured into the manometer. The results obtained by this method are recorded in table II.

Tissue Fragments.

Certain inoculations were made by inserting fragments of mosaic infected tissue into plants with a sterile scalpel. This

method is particularly suitable for field inoculations as a scalpel and an alcohol lamp for flaming are the only apparatus required. This method was used in the greenhouse in the earlier experiments. It is simpler than the hypodermic needle method as the scalpel may be sterilized by flaming just before each plant is inoculated and no provisions need be made for filtering juice for inoculum.

In making cross inoculations in the greenhouse by the tissue fragment method, it was the general practice to flame the scalpel just before inoculating each plant and the hands were in all cases thoroly washed with soap and water just before the inoculation of a series. In many cases the hands were washed before inoculating each individual plant of the series. Check plants were injured in a similar manner as were the inoculated plants, but in most cases no tissue was inserted into the checks.

Puncture Thru Inoculum.

Probably the most efficient method of artificially inoculating mosaic in cross inoculation investigations is to apply drops of pulpy mascerated mosaic tissue and juice to the plant and then prick the plant thru these drops with a sterile needle. To find out to what extent liquids thus inoculated will penetrate into the plant, drops of an aqueous solution of eosin were applied on the stems of young tomato plants and the plants were pricked thru these drops in a preliminary experiment. Immediately following the puncturing, the eosin solution was drawn down the stems for several centimeters and after a few hours the stain was drawn up the plant to the tip. By this method, then, the inoculum is efficiently injected into the inoculated plant. Mosaic tissue used as inoculum was mascerated in a sterile mortar and sufficient tap water was added to secure a rather liquid, pulpy inoculum which was transferred to the plants to be inoculated with the aid of a sterilized medicine dropper. The inoculum was applied at the points where it was desired to make inoculations and the plant was pricked a number of times thru these drops.

In addition to being an efficient method for transferring inoculum into the plant, this method reduces to a minimum the chances for accidental contamination. Mortars, pestles and medicine droppers were sterilized with heat and the needle was sterilized by flaming just before the inoculation of each plant. By this method the plant need not be touched by the operator. The drop of inoculum was placed at the desired points with the dropper and punctures were made with the needle. For the support of the leaves, tissue towel paper was used in some instances. These papers were folded over the leaf and thus support could be given without the operator's hands coming in contact with the plant. For

each plant an individual paper was used. Following the inoculation of a series the control plants were pricked thru drops of tap water.

Use of Acetone in Cross Inoculations.

Cross inoculations between certain species, as will be indicated later, were successful only when the inoculum was mascerated in a solution of acetone. In making these inoculations, newly developed leaves of the mosaic plants were mascerated in a sterile mortar in approximately four c.c. of 30 percent acetone in tap water.

Insects.

Insects are undoubtedly the most efficient agencies for transmission of the mosaic virus. Certain mosaic cross inoculations which were not successful when tried by other methods were secured thru the medium of insect vectors. Insect mosaic cross inoculation experiments in the greenhouse were not made in the mosaic free house, owing to the risk of some of these insects escaping. Plants were used in these investigations which had been propagated in the mosaic free greenhouse. The inoculations were made by infesting the plants with insects from a mosaic plant and keeping these plants in insect proof cages. The cages were either left covering the plants during the entire incubation period, or the plants, after having been infested for a sufficient time to give ample opportunity for infection, were taken from the cages and fumigated to kill the insects. After this the plants were placed in the mosaic free house until mosaic symptoms appeared.

When using mealy bugs (*Pseudococcus maritimus* Ehr.), as the means of transmitting mosaic infection, the plants, because of the difficulty in entirely ridding them of bugs, were left in the cages during the entire incubation period. In order to prevent the migration of insects to and from the plant, the pots were not only covered with fine screen cages, but also were either placed in shallow containers of water or a ring of tree tangle foot was applied around the outside of the pot.

EXPERIMENTAL DATA

Utilizing the methods described previously, numerous mosaic cross inoculation experiments were made among species belonging to different families and orders. The results obtained from these investigations follow. In the presentation of this data the hosts are grouped in their respective families.

INOCULATIONS AMONG SPECIES OF SOLANACEAE AND LEGUMINOSAE.

Mosaic cross inoculations were made from species of the Solanaceae to species of the Leguminosae and vice versa. Five species

TABLE I. CROSS INOCULATION AMONG SPECIES OF *SOLANACEAE* AND *LEGUMINOSAE*

Date of inoculation	Source of virus	Species inoculated	Methods‡	Results			Length of exp. in days
				No. inoculated	Infected	No.† checks	
1923		*Lycopersicon*					
3-15	*Phaseolus vulgaris*	*esculentum*	P	5	0	10	46
3-26	" "	" "	P*	5	4	10	35
6-11	" "	" "	P	5	0	10	35
10-17	" "	" "	P*	5	0	5	43
10-17	" "	" "	P	5	0	5	43
11-15	" "	" "	P*	5	0	5	37
11-15	" "	" "	P	5	0	5	37
2-28	" "	*Nicotiana tabacum*	TF	5	0	10	30
3-26	" "	" "	P*	5	4	13	35
5-24	" "	" "	P*	5	0	5	37
6-11	" "	" "	P*	5	0	5	34
10-17	" "	" "	P	5	0	5	46
10-17	" "	" "	P*	5	0	5	46
11-15	" "	" "	P*	5	0	5	37
11-15	" "	" "	P	5	0	5	37
3-26	" "	*Nicotiana alata*	P*	5	2	6	38
1922		*Lycopersicon*					
2-11	*Vigna sinensis*	*esculentum*	H	4	0	4	47
3-30	" "	" "	H	6	0	6	26
4- 5	" "	*Nicotiana tabacum*	TF	5	0	5	26
3-21	" "	*Solanum tuberosum*	TF	6	0	6	40
1923		*Lycopersicon*					
9-15	*Trifolium pratense*	*esculentum*	P*	7	0	7	56
9-15	" "	" "	P	7	0	7	56
9-15	" "	*Nicotiana tabacum*	P*	5	0	5	56
9-15	" "	" "	P	5	0	5	56
7-17	*Soja max*	" "	P*	10	0	10	30
7-17	" "	" "	P	5	0	14	30
3-15	*Lycopersicon esculentum*	*Phaseolus vulgaris*	TF	7	0	10	46
4- 5	" "	" "	TF	5	0	10	45
1922							
5-18	*Solanum tuberosum*	*Vigna sinensis*	A**	38	38	60	21
5-18	*Solanum melongena*	" "	MB***	36	28	60	21
6- 6	" "	" "	MB***	14	8	16	30
6- 6	" "	*Soja max*	MB***	14	0	11	30

*Inoculum macerated in acetone solution.
**Species undetermined.
****Pseudococcus maritimus Ehr.*
†No checks were infected.
‡P stands for puncture method. TF for tissue fragment, H for hypodermic needle, A for aphids and MB for mealy bugs.

of the Solanaceae, including tobacco *(Nicotiana tabacum);* tomato *(Lycopersicon esculentum);* potato *(Solanum tuberosum);* egg plant *(Solanum melongena),* and *Nicotiana alata* var. *grandiflora* were used in these experiments. In the Leguminosae four species were utilized, including bean *(Phaseolus vulgaris);* cow pea *(Vigna sinensis);* soy bean *(Soja max),* and red clover *(Trifolium pratense).*

SUMMARY OF TABLE I.

Artificial inoculations with mosaic bean tissue were made on March 26, 1923, to five plants each of tobacco, tomato and *Nicotiana alata.* The plants were inoculated with mosaic bean tissue, mascerated in a solution of 30 percent acetone.

Four tomato, four tobacco and two *N. alata* plants of this series became infected. The tomato plants exhibited mosaic symptoms in 25 to 26 days; two tobacco plants showed the symptoms in 28 days, one in 26 and one in 35 days; the two infected *N. alata* plants produced the first observable symptoms after 23 and 24 days, respectively. After making the mosaic inoculations the checks, which included 10 tomato, 13 tobacco and 6 *N. alata* plants, were pricked thru drops of acetone solution. All the checks remained healthy.

The above were the only trials where infections were secured from inoculations of mosaic bean tissue to species of Solanaceae altho a number of other attempts were made.

Artificial inoculations were made with the virus from mosaic cow pea to tomato, tobacco and to potato, and with mosaic virus from red clover to tomato and tobacco plants, but only negative results were obtained. In addition, mosaic infected soy bean tissue was inoculated artificially to tobacco plants without infection resulting.

Where insects served as transmitting agents, cow pea plants became infected with the mosaic virus from potato and from egg plant. Thirty-eight cow pea plants were infected on May 18, 1922, with aphids (species undetermined) from mosaic potato plants. All were infected at the end of 21 days, while the 60 check plants remained healthy. Mealy bugs *(Pseudococcus maritimus)* transferred on May 18, 1922, from mosaic infected egg plant to 36 cow pea plants, transmitted the mosaic disease to 28 of them within 21 days. The 60 check plants all remained healthy. On June 6, 1922, mealy bugs were transferred from mosaic infected egg plant to 14 cow pea plants. Eight exhibited mosaic symptoms after 19 days. Sixteen check plants were held, all of which remained healthy.

An attempt was made June 6, 1922, to transmit the mosaic virus from egg plant to soy bean plants thru the medium of mealy bugs. The mealy bugs colonized on the soy bean, but infection did not result. In addition to the results recorded in table I, many other attempts to transmit mosaic among species of Solanaceae and Leguminosae were negative but for the sake of brevity they are not included.

Artificial mosaic inoculations from Leguminosae to Solanaceae have resulted only when the inoculum was macerated in a solution of acetone. At present it is not known what effect acetone has in influencing mosaic transmission. Neither is it known that such transmission is impossible without the use of acetone. No infections resulted from artificial inoculations from species of Solanaceae to species of Leguminosae. Beans or cow peas have not been artificially infected with mosaic even tho the inoculum was from plants belonging to the same species.

Aphids and mealy bugs may serve as vectors in transmitting the mosaic virus from infected potatoes and egg plant to cow pea. These insects have facilitated mosaic infection to species (beans and cow peas) where artificial inoculations failed. The data obtained thru the use of these vectors, together with that from the artificial infections, indicate that mosaic is transmissible among species of Solanaceae and Leguminosae.

INOCULATIONS AMONG SPECIES OF SOLANACEAE AND CUCURBITACEAE.

A number of trials were made to secure cross infections with mosaic virus from species of Solanaceae to Cucurbitraceae and vice versa. Six species of Solanaceae, viz., tobacco *(Nicotiana tabacum),* tomato *(Lycopersicon esculentum),* petunia *(Petunia violacea),* potato *(Solanum tuberosum),* pepper *(Capsicum annuum)* and *Nicotiana rustica* and two species of Cucurbitaceae, cucumber *(Cucumis sativus)* and summer crook-neck squash *(Cucurbita pepo)* were used in this investigation.

SUMMARY OF TABLE II.

More than 60 tobacco plants (44 of these are listed in table II) were inoculated with the mosaic virus from cucumber and infection resulted in 18 of these. The length of the incubation period varied from 12 to 25 days. Attempts to transfer mosaic infection from tobacco to cucumber were not successful.

Five tobacco plants were inoculated on October 31, 1923, with mosaic cucumber tissue mascerated in acetone and five plants were inoculated with the mosaic tissue macerated in water. All of the plants in both series became infected and from this it is evident that the acetone was of no assistance in effecting this cross. Ten plants were held as checks and all remained healthy.

Three trials were made to transmit the mosaic virus from cucucumber to petunia. Infection resulted in 4 of the 10 plants that were inoculated. Three petunia plants inoculated on December 22, 1921, with mosaic cucumber tissue became infected in 26 days and one of the five plants inoculated on March 21, 1922, exhibited mosaic symptoms in 12 days. An equal number of checks were held in these experiments and all remained healthy.

Artificial cross infection with mosaic virus was secured from summer crookneck squash to tobacco, tomato and petunia; and reciprocally the mosaic virus from tobacco and tomato was artificially transmitted to crookneck squash.

Fifty-seven tobacco plants were inoculated with mosaic virus from crookneck squash and of these 10 became infected. Infection resulted in one tobacco plant, following the injection of filtered juice from mosaic crookneck squash under pressure

TABLE II. CROSS INOCULATION AMONG SPECIES OF *SOLANACEAE* AND *CUCURBITACEAE*

Date of inoculation	Source of virus	Species inoculated	Methods‡	Results: No. inoculated	Results: Infected	Results: No.† checks	Length of exp. in days
1921							
12-22	*Cucumis sativus*	*Nicotiana tabacum*	TF	2	0	2	22
1922							
4-5	" "	" "	TF	5	1	30	25
5-31	" "	" "	TF	12	1	12	27
12-15	" "	" "	TF	4	4	5	36
1923							
5-24	" "	" "	P*	5	1	5	23
6-15	" "	" "	P	5	1	10	19
10-31	" "	" "	P	5	5	5	25
10-31	" "	" "	P*	5	5	5	25
1922							
7-12	" "	*Lycopersicon esculentum*	TF	60	0	60	49
7-13	" "	" "	TF	60	0	60	48
1921							
12-22	" "	*Petunia violacea*	TF	3	3	3	22
1922							
2-24	" "	" "	TF	2	0	5	35
3-21	" "	" "	H	5	1	5	40
3-21	" "	*Solanum tuberosum*	TF	6	0	6	40
7-13	" "	*Capsicum annuum*	TF	5	0	5	48
7-14	" "	" "	TF	60	0	60	47
1921							
12-27	*Cucurbita pepo*	*Nicotiana tabacum*	TF	2	0	3	17
1922							
2- 3	" "	" "	MP	2	1	6	45
3-27	" "	" "	H	5	2	5	30
4- 5	" "	" "	TF	5	5	30	25
4-11	" "	" "	MB***	5	2	5	34
5- 2	" "	" "	TF	8	0	8	44
5- 4	" "	" "	H	10	0	10	23
7-11	" "	" "	TF	20	0	20	50
1921		*Lycopersicon*					
12-27	" "	*esculentum*	TF	4	0	4	30
1922							
3-25	" "	" "	MP	5	1	10	29
3-27	" "	" "	H	5	2	10	28
4-11	" "	" "	MB***	5	1	5	34
7-11	" "	" "	TF	10	0	10	50
1923							
2-27	" "	" "	H	2	0	2	37
1922							
2-11	" "	*Petunia violacea*	TF	5	4	5	44
3-27	" "	" "	H	2	0	2	39
7-11	" "	" "	TF	5	0	5	50
3-24	" "	*Solanum tuberosum*	H	10	0	10	35
3-27	" "	*Solanum melongena*	H	4	0	10	29
1923							
2-27	" "	" "	H	2	0	2	37
1922							
3-30	" "	*Capsicum annuum*	H	10	0	10	46
7-11	" "	*Nicotiana rustica*	TF	5	0	5	50
3-31	*Nicotiana tabacum*	*Cucumis sativus*	H	14	0	17	31
1923							
3-25	" "	" "	MB***	2	0	2	30
7-16	" "	*Cucurbita pepo*	P	10	0	10	31
1922							
2- 3	" "	" "	H	4	0	15	45
2- 3	" "	" "	MP	4	4	15	45
3-30	" "	" "	H	10	0	10	45
1923							
3-25	" "	" "	MB***	1	0	1	30
1922	*Lycopersicon*						
2- 3	*esculentum*	" "	MP	4	4	15	45
1923							
3-31	" "	" "	MB***	1	0	1	30
3-31	*Solanum tuberosum*	" "	P	3	0	3	30
1922							
5- 6	" "	*Cucumis sativus*	A**	12	0	8	34

*Inoculum macerated in acetone solution.
**Species undetermined.
***_Pseudococcus maritimus_ Ehr.
†No checks were infected.
‡TF stands for tissue fragment, P for puncture, H for hypodermic needle, MP for manometric pressure, MB for mealy bugs and A for aphids.

produced by a mercury column. The incubation period was 20 days. Two of the five tobacco plants that were inoculated by hypodermic needle with the mosaic virus from crookneck squash on March 27, 1922, developed infection in 21 days. Five tobacco plants were infested with mealy bugs from infected crookneck squash plants on April 11, 1922, of which two developed mosaic in 30 days.

Mosaic virus from tobacco was inoculated to 29 crookneck squash plants of which four became infected. Fifty-one check plants were held and all remained healthy. The four plants that became infected were inoculated by forcing into them filtered juice from mosaic tobacco tissue under pressure produced by a mercury column. These plants exhibited mosaic symptoms at the end of 30 days.

A total of 31 tomato plants were inoculated with mosaic virus from summer crookneck squash and of these four became infected. The 41 plants held as checks all remained healthy. In an experiment on March 25, 1922, five tomato plants were inoculated with mosaic juice from crookneck squash under pressure of a mercury column. One of these developed mosaic symptoms in 20 days. On March 27, 1922, five tomato plants were inoculated with juice from mosaic crookneck squash by the hypodermic needle method and two of these showed symptoms at the end of 21 days. Mealy bugs transferred from mosaic crookneck squash plants to five tomato plants transmitted the disease to one of these, the incubation period being 30 days.

Mosaic tomato juice was inoculated on February 3, 1922, to four crookneck squash plants under pressure produced by a mercury column. All of these plants exhibited mosaic symptoms at the end of 30 days. The 15 checks remained healthy.

Three trials were made to transmit artificially mosaic infection from crookneck squash to petunia. Five petunia plants were inoculated on February 11, 1922, by the tissue fragment method. Four of these developed the mosaic disease. Two additional attempts to transmit mosaic infection from crookneck squash to petunia were unsuccessful. A total of 12 checks were held and all remained healthy.

Unsuccessful attempts were made to transmit mosaic from crookneck squash to other species of Solanaceae. Inoculations were made to 10 potato, 6 egg plant, 5 *Nicotiana rustica,* and to 10 pepper plants. Negative results were also obtained from the inoculation of three crookneck squash plants with juice from mosaic potato.

The data presented above indicate that the mosaic virus was transmitted from cucumber to tobacco and to petunia; from crookneck squash to tobacco and to tomato and vice versa, and

from crookneck squash to petunia. A majority of these infections followed artificial inoculations but a limited number of cross infections were secured thru the medium of mealy bugs. These data indicate that the mosaic disease is transmissible among certain species of the Solanaceae and the Curcurbitaceae.

INOCULATIONS AMONG SPECIES OF LEGUMINOSAE AND CUCURBITACEAE.

Mosaic cross inoculations between species of Leguminosae and Cucurbitaceae were attempted both artificially and thru the medium of insect vectors. Two species of the Leguminosae, bean *(Phaseolus vulgaris)* and cow pea *(Vigna sinensis)*, and two species of Cucurbitaceae, cucumber *(Cucumis sativus)* and summer crookneck squash *(Cucurbita pepo* var. *condensa)* were used in these experiments.

SUMMARY OF TABLE III.

In experiments to transmit the mosaic virus from cucumber to cow pea, 27 cow pea plants were inoculated. Eleven of these plants were inoculated by the hypodermic needle method, but infections did not result. *Aphis gossypii* were transferred on November 30, 1923, from mosaic infected cucumber plants to six cow pea plants with the result that three of the cow pea plants became infected in 14 days. Fifteen plants which were held as checks remained healthy. Mealy bugs were transferred from mosaic infected cucumbers to 10 cow pea plants on February 26, 1923, but infections did not result. Likewise, negative results were obtained in a similar attempt on February 28, 1923, to transmit mosaic from bean to three cucumber plants.

In February, 1922, there were present in one of the horticultural greenhouses mosaic crookneck squash plants that were heavily infested with mealy bugs. Adjoining these crookneck

TABLE III. CROSS INOCULATION AMONG SPECIES OF *LEGUMINOSAE* AND *CUCURBITACEAE*

Date of inoculation	Source of virus	Species inoculated	Methods‡	Results			Length of exp. in days
				No. inoculated	Infected	No.† checks	
1922 3-30	*Cucumis sativus*	*Vigna sinensis*	H	11	0	45	31
1923 2-16	" "	" "	MB*	10	0	10	42
11-30	" "	" "	AG	6	3	15	17
1922 3-30	*Cucurbita pepo*	" "	MB*	16	16	11	15
4- 8	" "	" "	MB*	32	20	21	15
1923 2-28	*Phaseolus vulgaris*	*Cucumis sativus*	MB*	3	0	10	30

**Pseudococcus maritimus* Ehr.
†No checks were infected.
‡H stands for hypodermic needle, MB for mealy bugs and AG for aphis gossypii.

squash plants there stood a flat of cow pea seedlings. Mosaic infection was noticed first on some of the cow peas when they were approximately seven inches high. Subsequent examination showed that they were becoming infested with mealy bugs. Mosaic infection occurred first in plants growing on the side of the flat adjacent to the mosaic crookneck squash plants and the initial mealy bug infestation of the cowpeas was on this side of the flat.

Later, cow pea plants under controlled conditions, were infested with mealy bugs from the mosaic crookneck squash plants to learn definitely, first, whether the mosaic virus is transmissible from the crookneck squash to the cowpea and, second, if mealy bugs serve as vectors of the mosaic causal agent. On March 30, 1922, 16 cow pea plants were infested with mealy bugs from mosaic crookneck squash plants and an equal number of cow pea plants were held as checks. The 16 plants infested with the mealy bugs developed the mosaic disease in 14 days, while the checks all remained healthy. A second attempt to transmit mosaic from crookneck squash to cow peas thru the medium of mealy bugs was made April 8, 1922. In this trial 32 cow pea plants were infested with the mealy bugs and 21 plants were held as checks. Twenty of the infested plants exhibited mosaic symptoms within 17 days. The checks remained healthy.

Altho many attempts were made to transfer mosaic artificially from the Cucurbitaceae to the Leguminosae, no positive results were obtained. When insect vectors were used (mealy bugs) the transfer was effected from crookneck squash to cow peas in three of four trials.

INOCULATIONS AMONG SPECIES OF COMPOSITAE AND SOLANACEAE.

Mosaic was found on four species of the Compositae that have not been reported as hosts of this disease. These species include *Zinnia elegans, Calendula officinalis, Heliopsis scabra* and *Stokesia laevis.* The symptoms exhibited on these infected plants were the typical mosaic mottling which was especially evident on the leaves near the growing point of the stems. Similar symptoms were found on plants of *Lactuca scariola, Vernonia fasciculata* and *Verbena stricta,* but the infectiousness of their juices has not been demonstrated.

Cross inoculations were made from mosaic plants of zinnia, calendula and *Stokesia laevis* to tobacco *(Nicotiana tabacum)* and to tomato *(Lycopersicon esculentum).* The results obtained from these inoculations are summarized in table IV.

SUMMARY OF TABLE IV.

Five tobacco and five tomato plants were inoculated on October 10, 1922, with the mosaic virus from zinnia. Three of

TABLE IV. CROSS INOCULATION AMONG SPECIES OF *SOLANACEAE* AND *COMPOSITAE*

Date of inoculation	Source of virus	Species inoculated	Methods‡	Results			Length of exp. in days
				No. inoculated	Infected	No.† checks	
1922							
10-10	*Zinnia elegans*	*Nicotiana tabacum*	TF	5	3	5	48
1923							
10-18	" "	" "	P*	5	0	5	65
10-18	" "	" "	P	5	1	5	65
1922							
10-10	" "	*Lycopersicon esculentum*					
1923	" "		TF	5	5	5	54
10-18	" "	" "	P*	5	0	5	65
10-18	" "	" "	P	5	0	5	65
1922							
11-27	*Calendula officinalis*	*Nicotiana tabacum*	TF	6	4	9	25
11-27	" "	*Lycopersicon esculentum*					
1923			TF	6	1	16	25
9-15	*Stokesia laevis*	" "	P*	7	0	7	45
9-15	" "	" "	P	7	0	7	45
9-15	" "	*Nicotiana tabacum*	P*	5	2	5	45
9-15	" "	" "	P	5	1	5	45
6-14	*Heliopsis scabra*	*Heliopsis scabra*	A	3	3	20	20
3- 1	*Lycopersicon esculentum*	*Zinnia elegans*	TF	9	0	9	24
3- 6	" "	" "	TF	6	0	8	24
3- 1	*Nicotiana tabacum*	" "	TF	5	0	5	24
3- 6	" "	" "	TF	5	0	7	29
4- 7	" "	" "	TF	5	0	11	20

*Inoculum macerated in acetone solution.
†No checks were infected excepting one of the nine in the cross inoculation 11-27-22 from *Calendula officinalis* to *Nicotiana tabacum*.
‡TF stands for tissue fragment, P for puncture and A for aphids.

the tobacco plants and all of the tomato plants became infected and exhibited mosaic symptoms after an incubation period of from 17 to 26 days. The five tobacco and five tomato plants that were held as checks remained healthy. On October 18, 1923, 10 tobacco and 10 tomato plants were inoculated with mosaic zinnia tissue. These inoculations were made with the mosaic tissue macerated both in acetone and in water. One tobacco plant that was inoculated with the zinnia tissue macerated in water exhibited infection in 16 days. An equal number of checks and the remaining inoculated plants all remained healthy.

Mosaic calendula tissue was inoculated on November 27, 1922, to six tobacco and to six tomato plants. Four of the tobacco and one of the tomato plants became infected and exhibited mosaic symptoms from 15 to 20 days following the inoculations. Nine tobacco and 16 tomato plants were held as checks and all remained healthy.

Juice from a mosaic affected *Stokesia laevis* plant was inoculated on September 15, 1923, to 10 tobacco and 14 tomato plants. Acetone was used in macerating the mosaic tissue inoculated to five of the tobacco and to seven of the tomato

plants. Infection resulted to tobacco both in the series where acetone was or was not used. Three of the tobacco plants became infected, the mosaic symptoms being exhibited in 24, 27 and 28 days following the inoculation. The tomato plants were not infected and an equal number of checks, both of tobacco and tomato, remained healthy.

Thirty zinnia plants were inoculated with mosaic inoculum from tobacco and tomato, but infections were not obtained.

Plants of *Heliopsis scabra* that had been propagated from seed in one of the greenhouses in 1923 were found infected with the mosaic disease in 1923. Approximately 65 plants were being propagated of which 17 were affected with this disease. The affected plants not only exhibited mosaic mottling, but were severely stunted. These plants were removed to the greenhouse, which was being used for the propagation of mosaic plants, and aphids (species undetermined) were there colonized on them. Three healthy plants of *Heliopsis scabra* were infested on June 14, 1923, with aphids from the mosaic Heliopsis plants and all became infected within 18 days.

Evidence has been presented that four species of Compositae not heretofore reported subject to mosaic, are affected with this disease. Infections have been secured from mosaic to healthy plants of *Heliopsis scabra* thru the medium of aphids. Mosaic tissue from zinnia and calendula was artificially transmitted to tomato; mosaic infection was secured from zinnia, from calendula and from *Stokesia laevis* to tobacco. The results obtained indicate that the mosaic virus is transmissible from species of the Compositae to species of the Solanaceae.

INOCULATIONS AMONG SPECIES OF MONOCOTYLEDONEAE AND DICOTYLEDONEAE.

The successful transmission of mosaic among families and orders of the Dicotyledoneae has indicated that this disease is interter-transmissible among species of plants that are widely separated taxonomically. The possibility was suggested that mosaic transmission might result among species of the Monocotyledoneae and the Dicotyledoneae. Cross inoculation experiments were carried on to determine if such cross infections were possible. Mosaic infected sugar cane (*Saccharum officinarum* var. Demerara No. 74) plants were obtained from Louisiana. These plants were propagated in the greenhouse at Ames and served as a source for sugar cane mosaic inoculum.

Attempts were made to inoculate mosaic virus from infected sugar cane and corn *(Zea mays)* to six species of the Dicotyledoneae; namely, tobacco *(Nicotiana tabacum);* tomato *(Lycopersicon esculentum);* jimson weed *(Datura stramonium);* cucumber *(Cucumis sativus);* summer crookneck squash *(Cucur-*

TABLE V. CROSS INOCULATIONS AMONG SPECIES OF *MONOCOTYLEDONAE* AND *DICOTYLEDONAE*

Date of inoculation	Source of virus	Species inoculated	Methods‡	Results			Length of exp. in days
				No. inoculated	Infected	No.† checks	
1923	*Saccharum*						
4-15	*officinarum*	*Nicotiana tabacum*	P*	5	2	30	30
6-11	" "	" "	P*	5	0	10	48
6-11	" "	" "	P	5	0	5	48
7-16	" "	" "	P*	5	0	15	40
7-16	" "	" "	P	5	0	15	40
7-18	" "	" "	P*	10	4	10	29
7-18	" "	" "	P	5	0	4	21
9-14	" "	" "	P*	5	3	5	58
9-14	" "	" "	P	5	0	5	58
10-11	" "	" "	P*	5	0	5	60
10-11	" "	" "	P	5	0	5	60
11-26	" "	" "	P*	5	0	5	26
11-28	" "	" "	P*	10	0	10	24
11-28	" "	" "	P	5	0	5	24
5-11		*Lycopersicon esculentum*	P*	5	2	5	40
	" "	" "	P*	5	3	10	40
6-16	" "	" "	P	5	0	10	40
6-16	" "	" "	P*	7	3	7	58
9-14	" "	" "	P	7	0	7	58
9-14	" "	*Datura stramonium*	P*	5	0	5	24
12-28	" "	" "	P	5	0	5	24
12-28	" "	*Cucumis sativus*	P*	40	0	50	33
7-18	" "	" "	P	20	0	20	33
7-18	" "	" "	P*	5	0	5	58
9-14	" "	" "	P	5	0	5	58
9-14	" "	*Cucurbita pepo condensa*	P*	2	0	2	33
7-18	" "	" " (pumpkin)	P*	2	0	2	33
7-18	" "	*Nicotiana tabacum*	P*	5	1	5	46
9-14	*Zea mays*	" "	P	5	0	5	46
9-14	" "	*Lycopersicon esculentum*	P*	5	0	5	46
9-15	" "	" "	P	5	0	5	46
9-15	" "	*Cucumis sativus*	P*	5	0	5	46
9-15	" "	" "	P	5	0	5	46
9-15	" "	" "	P	5	0	5	46
10-25	*Nicotiana tabacum*	*Achyrodes aureum*	P*	5	0	5	57
10-25	" "	" "	P*	5	0	5	57
10-25	" "	*Digitaria sanguinales*	P*	3	0	3	57
10-25	" "	" "	P*	3	0	3	57
10-25	" "	*Setaria glauca*	P*	3	0	3	57

*Inoculations macerated in acetone.
†No checks were infected.
‡P stands for puncture.

bita pepo var. *condensa*), and sugar pie pumpkin *(Cucurbita pepo).*

SUMMARY OF TABLE V.

Successful attempts were made to transmit mosaic infection from sugar cane to tobacco and to tomato. A total of 80 tobacco plants were artificially inoculated with mosaic sugar cane tissue. Nine of these plants became infected. One hundred twenty-nine checks were held and all remained healthy. Mosaic infection from sugar cane to tobacco resulted only in plants where the inoculum was macerated in acetone solution.

Five tobacco plants were inoculated on April 15, 1923, with mosaic inoculum from sugar cane. Two of these developed the

disease in 24 days. Five tobacco plants were inoculated in a similar manner on September 14, 1923, and three of these became infected with incubation periods of 27, 30 and 31 days, respectively. Inoculations were made on July 18, 1923, with mosaic sugar cane tissue to 15 tobacco plants that were growing in the field. Ten of the 15 plants were inoculated with the infected tissue that had been macerated in acetone solution; four developed mosaic symptoms at the end of 21 days. The remaining five were inoculated with the infected sugar cane tissue macerated in water and these with the 14 checks remained healthy. Although the plants used in this experiment were not caged the results obtained are nevertheless of value owing to the fact that the checks with which the inoculated plants were alternated in the row all remained healthy.

Mosaic infected sugar cane tissue was inoculated to 29 tomato plants of which 8 developed infection. Thirty-nine tomato plants were held as checks and all remained healthy. As was the case in the transmission of mosaic from sugar cane to tobacco, infection of tomato plants resulted only in cases where the inoculum was macerated in a solution of acetone.

Five tomato plants were inoculated on May 11, 1923, with mosaic sugar cane tissue macerated in acetone. Two of these developed mosaic disease in 22 days. Five plants were similarly inoculated on June 16, 1923, and of these, three developed infection. The incubation period of two of these plants was 31 days, while the third plant exhibited mosaic symptoms in 34 days. On September 14, 1923, seven tomato plants were inoculated with mosaic sugar cane tissue macerated in acetone. Three of these became infected, of which two exhibited mosaic symptoms in 25 days and one in 29 days.

Attempts were made to transmit mosaic from sugar cane to 10 jimson weeds, 70 cucumbers, 2 summer crookneck squashes, and to 2 pie pumpkins, but infection did not result.

An attempt was made on September 14, 1923, to transmit mosaic infected corn tissue to tobacco. Ten plants were inoculated of which one became infected. This plant was inoculated with the mosaic corn tissue macerated in acetone solution.

Trials made to transmit mosaic artificially from tobacco to three species of grasses, including *Digitaria sanguinalis*, *Achyrodes aurem* and *Setaria glauca* were negative.

The infections that were obtained as a result of mosaic inoculations from sugar cane and corn to tobacco and tomato were in every case plants that were inoculated with the mosaic tissue macerated in a solution of acetone. A sufficient number of inoculations have not been made to exclude the possibility that such cross infection may not result without the use of acetone.

Mosaic infection was transmitted from sugar cane to tobacco and tomato and in one case the mosaic virus from corn was carried successfully to tobacco. These results indicate that mosaic virus is transmissible at least from certain species of the Monocotyledoneae to certain species of the Dicotyledoneae.

TRANSMISSIBILITY OF MOSAIC FROM EIGHT OTHER SPECIES.

The majority of species known to be susceptible to mosaic occur in the Solanaceae, Cucurbitaceae, Leguminosae, Compositae, and Gramineae. However, mosaic has been reported on species in 18 other families. The results of cross inoculation investigations that were made with eight of these species are recorded in the following paragraphs.

Mosaic of Apium graveolens

Mosaic infected celery plants *(Apium graveolens)* were found in August, 1923, in the Experiment Station gardens. In the case of a few plants mottling of the leaves was evident; in addition it was noticed that many of the plants besides being small and stunted produced abnormally spindling or filiform leaves with narrow elongated lobes. Three plants with only the filiform leaf symptoms were potted and placed in the greenhouse. Later in the fall the leaves produced on these plants exhibited the characteristic mosaic mottling.

Attempts were made to transmit mosaic infection from celery to tobacco *(Nicotiana tabacum);* tomato *(Lycopersicon esculentum);* jimson weed *(Datura stramonium);* cucumber *(Cucumis sativus),* and cow pea *(Vigna sinensis).* These inoculations were made artificially and thru the medium of *Aphis gossypii.*

SUMMARY OF TABLE VI.

Ten tobacco plants were inoculated September 15, 1923, with mosaic virus from celery. Five became infected after incubation periods of 20 to 25 days. Five of the 10 plants were inoculated with mosaic celery tissue macerated in a solution of acetone, while the remaining five were inoculated with the celery tissue macerated in water. Infections resulted in both series. An equal number of checks were held, all of which remained healthy. On November 16, 1923, mosaic celery tissue macerated in water was inoculated to five tobacco plants. Four of these plants exhibited mosaic symptoms in 16 days. The five checks remained healthy. A third attempt was made December 3, 1923, to infect tobacco with mosaic from celery. The inoculations were made in the same way as those described under date of November 16. The five plants inoculated exhibited mosaic symptoms in 14 days. Inoculations with mosaic infected celery were made on March 20, 1924, to a fourth series of tobacco plants. Five tobacco

TABLE VI. CROSS INOCULATION TRIALS WITH THE MOSAIC VIRUS FROM *APIUM GRAVEOLENS*

Date of inoculation	Source of virus	Species inoculated	Methods‡	Results			Length of exp. in days
				No. inoculated	Infected	No.† checks	
1923 9-15	*Apium graveolens*	*Nicotiana tabacum*	P*	5	3	5	35
9-15	" "	" "	P	5	2	5	35
11-16	" "	" "	P	5	4	5	36
12- 3	" "	" "	P	5	5	5	20
1924 3-20	" "	" "	P	5	2	5	22
1923 9-15	" "	*Lycopersicon esculentum*	P*	7	1	7	35
9-15	" "	" "	P	7	2	7	35
1924 3-20	" "	" "	P	5	4	5	22
1923 12- 3	" "	*Datura stramonium*	P	5	0	5	21
12-21	" "	*Cucumis sativus*	A**	2	2	2	14
12-26	" "	" "	A**	13	10	10	17
1924 1-15	" "	" "	A**	10	8	10	14
3-20	" "	" "	P	10	1	10	28
1923 12-21	" "	*Vigna sinensis*	A**	6	0	7	39
1924 1-15	" "	" "	A**	10	0	10	34

*Inoculum macerated in acetone solution.
**_Aphus gossypii_.
†None of the checks were infected.
‡P stands for puncture and A for aphids.

plants were inoculated with the celery tissue macerated in water. Two of the inoculated plants developed infection in 14 days, while the five checks remained healthy.

Inoculations with mosaic from celery have been made to two series of tomato plants. Fourteen tomato plants were inoculated with mosaic celery tissue, September 15, 1923. The inoculum used for seven of these was macerated in acetone solution and the inoculum for the remaining seven was macerated in water. One of the plants inoculated with the inoculum in acetone exhibited mosaic symptoms in 19 days and two of the plants inoculated with the inoculum in water exhibited such symptoms in 18 days. The 14 checks that were held all remained healthy. On March 20, 1924, a second attempt was made to infect tomato plants with mosaic from celery. Of five plants that were inoculated, four became infected and exhibited mosaic symptoms in 14 days. The five checks remained healthy.

An attempt was made on December 3, 1923, to infect five jimson weed plants with mosaic from celery but infections did not result.

*Aphis gossypii** were found in December, 1923, infested on the mosaic celery plants in the greenhouse. Three successful attempts were made to utilize these aphids as a medium for

*Determined by Dr. E. M. Patch, Maine Agr. Exp. Sta.

transmitting mosaic infection from celery to cucumber plants. A preliminary experiment was made on December 21, 1923, to determine if the aphids from the celery plants would colonize on caged cucumbers. Aphids were transferred to two cucumber plants. Infestation of the cucumbers resulted and in 20 days both plants were mosaic. Two control plants were held, both of which remained healthy. Thirteen cucumber plants were infested on December 26, 1923, with *Aphis gossypii* from mosaic celery plants. Ten of these exhibited mosaic symptoms in 16 days and an equal number of checks all remained healthy. A third attempt to transmit mosaic from celery to cucumbers thru the medium of *Aphis gossypii* was made on January 15, 1924. Ten cucumber plants were infested with the aphids. Eight of these plants exhibited mosaic symptoms in 14 days of which two showed symptoms in 9 days. The 10 checks remained healthy. Mosaic celery juice was artificially inoculated on March 20, 1924, to 10 cucumber plants and an equal number of checks were held. One of the infected plants developed mosaic symptoms in 20 days while the checks all remained healthy.

Two attempts were made to transmit mosaic infection from celery to cow peas thru the agency of *Aphis gossypii.* These aphids were transferred to 16 cow pea plants but infections did not result. The aphids soon died and it is not known that they had fed on the cow peas.

The results above cited show that mosaic was transmitted from celery to tobacco, to tomato and to cucumber. These infections were secured both thru artificial inoculations and by means of *Aphis gossypii* as a medium for transmission. *Aphis gossypii* has been found to utilize celery as a host plant and has served in transferring mosaic infection between this species and cucumber. As a result of the cross infections that were secured, evidence is presented that the mosaic disease is transmissible from the Umbelliferae to the Solanaceae and Cucurbitaceae.

Mosaic of Rubus strigosus

Raspberry *(Rubus strigosus)* in Iowa was observed to be exhibiting a diseased condition, the symptoms of which suggested the mosaic disease. It has, however, not been proven by inoculations that this disease is infectious. A number of mosaic raspberry plants (variety unknown) were obtained from Ontario, Canada, and were grown in the greenhouse at Ames. Leaves of these plants produced a very characteric mosaic mottling. The mosaic-like raspberries from Iowa, even when grown in the greenhouse, have not produced a similar mottling to that produced on the plants obtained from Canada. It may be that this difference of mottling is due to a comparison of the mosaic disease on different varieties.

TABLE VII. CROSS INOCULATION TRIALS WITH THE MOSAIC VIRUS FROM *RUBUS STRIGOSUS*

Date of inoculation	Source of virus	Species inoculated	Methods‡	Results			Length of exp. in days
				No. inoculated	Infected	No.† checks	
1922							
12-15	*Rubus strigosus*	*Nicotiana tabacum*	TF	4	3	5	26
1923							
2-19	" "	" "	TF	4	0	4	22
2-25	" "	" "	TF	5	0	10	28
9-14	" "	" "	P*	5	1	5	26
9-14	" "	" "	P	5	3	5	26
12-25	" "	*Lycopersicon esculentum*	TF	12	0	15	29
3- 2	" "	" "	TF	8	0	15	56
9-14	" "	" "	P*	7	0	7	57
9-14	" "	" "	P	7	0	7	57
2-28	" "	*Solanum melongena*	TF	4	0	9	28
3- 2	" "	*Cucumis sativus*	TF	4	0	10	56
2-28	" "	*Cucurbita sp.*	TF	7	0	6	28
2-28	" "	*Zinnia elegans*	TF	9	0	9	28
1921							
12-23	*Nicotiana tabacum*	*Rubus strigosus*	TF	2	0	2	40

*Inoculum macerated in acetone solution.
†None of the checks were infected.
‡TF stands for tissue fragment and P for puncture.

Attempts were made to transmit mosaic from the infected raspberries obtained from Canada to tobacco *(Nicotiana tabacum;* tomato *(Lycopersicon esculentum);* egg plant *(Solanum melongena);* cucumber *(Cucumis sativus);* gourd *(Cucurbita sp.),* and to *Zinnia elegans.*

SUMMARY OF TABLE VII.

Two successful attempts were made to transmit mosaic from raspberry to tobacco. The first of these was made on December 15, 1922, when four tobacco plants were inoculated. Three of these became infected, one developing mosaic symptoms in 20 days and the other two in 26 days. Five checks were kept and all remained healthy. Ten tobacco plants were inoculated on September 14, 1923, with mosaic raspberry juice. Five were injected with inoculum where acetone was used in the maceration and for the remaining five plants the inoculum was macerated in water. Infections resulted in both series and four of the ten plants developed the mosaic disease. The incubation periods of these four plants were from 18 to 24 days. The ten checks remained healthy.

Unsuccessful attempts were made to transmit mosaic infection from raspberry to 34 tomato, 4 cucumber, 7 gourd, 9 zinnia and to 4 egg plants.

Cross inoculation experiments indicate that the mosaic disease is transmissible from raspberry to tobacco. Seven of the 23 tobacco plants that were inoculated became infected. Attempts to

TABLE VIII. CROSS INOCULATION TRIALS WITH THE MOSAIC VIRUS FROM *ASCLEPIAS SYRIACA*

Date of inoculation	Source of virus	Species inoculated	Methods‡	Results			Length of exp. in days
				No. inoculated	Infected	No.† checks	
1922							
12- 4	*Asclepias syriaca*	*Nicotiana tabacum*	TF	4	2	15	33
1923							
9-15	" "	" "	P*	5	0	5	56
9-15	" "	" "	P	5	3	5	56
1922		*Lycopersicon*					
10-10	" "	*esculentum*	TF	5	3	5	48
1923							
9-15	" "	" "	P*	7	0	7	56
9-15	" "	" "	P	7	0	7	56
6-20	" "	*Asclepias syriaca*	TF	3	2	5	30

*Inoculum macerated in acetone solution.
†None of the checks were infected.
‡TF stands for tissue fragment and P for puncture.

transmit raspberry mosaic to other species were not successful. While the results obtained are not extensive the evidence obtained indicated that the mosaic virus is transmissible from species of the Rosaceae to the Solanaceae.

Mosaic of Asclepias syriaca.

Milkweed *(Asclepias syriaca)* has frequently been found in Iowa infected with the mosaic disease. Artificial cross inoculations were made from mosaic milkweed to tobacco *(Nicotiana tabacum)* and to tomato *(Lycopersicon esculentum)*.

SUMMARY OF TABLE VIII.

Four tobacco plants were inoculated on December 4, 1922, with milkweed mosaic. Two plants became infected, the mosaic symptoms being evident on these at 31 and 33 days, respectively, following the inoculations. Fifteen check plants were held, all of which remained healthy.

Inoculations were made with mosaic from milkweed to five tobacco plants on September 15, 1923. Three developed mosaic symptoms in 24 to 28 days following the inoculations. The five checks all remained healthy.

Ten tomato plants were inoculated with mosaic milkweed tissue on October 10, 1922, and three of these became infected within 21 days following the inoculations. Ten checks were held and all remained healthy.

In the above experiments, 5 tobacco plants of the 14 that were inoculated and 3 tomato plants of the 19 that were inoculated became infected. These successful cross infections give evidence that mosaic is transmissible from Asclepidiaceae to Solanaceae.

TABLE IX. CROSS INOCULATION TRIALS WITH THE MOSAIC VIRUS FROM *MARTYNIA LOUISIANA*

Date of inoculation	Source of virus	Species inoculated	Methods‡	Results			Length of exp. in days
				No. inoculated	Infected	No.† checks	
1923							
4- 4	*Martynia louisiana*	*Nicotiana tabacum*	P	6	2	5	37
4- 4	" "	*Lycopersicon esculentum*	P	5	4	5	37
1922							
10-10	" "	" "	TF	5	4	5	36
11- 2	*Nicotiana tabacum*	*Martynia louisiana*	TF	4	3	6	46
1923							
2- 7	" "	" "	P	2	2	2	44
7- 3	" "	" "	TF	5	2	5	44
7-16	" "	" "	TF	17	6	21	31

†None of the checks were infected.
‡P stands for puncture and TF for tissue fragment.

Mosaic of Martynia louisiana.

In the summer of 1922 a plant of *Martynia louisiana* was found in the field infected with mosaic disease. From the fact that in the vicinity of this Martynia there were numerous mosaic infected plants of the Solanaceae, it was suspected that infection had come from these. Cross inoculation experiments were made to secure data on the question of the transmissibility of mosaic from *Martynia louisiana* to species of the Solanaceae and vice versa. Tobacco *(Nicotiana tabacum)* and tomato *(Lycopersicon esculentum)* were used for these investigations.

SUMMARY OF TABLE IX.

Five tobacco plants were inoculated with mosaic Martynia tissue on April 4, 1923. Two of the tobacco plants showed mosaic mottling in 19 days. An equal number of checks were held and these remained healthy.

Four of five tomato plants that were inoculated with mosaic Martynia juice on April 4, 1923, became infected. The incubation period was from 19 to 22 days. A second attempt was made on October 10, 1922, to transmit mosaic from Martynia to tomato. Five plants were inoculated, of which four became infected. The inoculation period was 14 days. Five checks were held and all remained healthy.

Four attempts were made to transmit mosaic from tobacco to Martynia and infection resulted in all of these. Twenty-eight Martynia plants were used in these four attempts and 13 plants exhibited mosaic symptoms after incubation periods varying from 19 to 34 days. A total of 34 checks were held and all remained healthy.

The evidence presented offers proof that mosaic is transmissible among species belonging to the Martyniaceae and the Solonaceae. Of the 43 plants that were inoculated 23 became infected.

Mosaic of Nepeta cataria.

During the winter of 1921-22 a number of catnip *(Nepeta cataria)* plants were growing in one of the college greenhouses. Growing adjacent to these plants were a number of mosaic crookneck squash plants that were heavily infested with mealy bugs. Mealy bugs were observed feeding on the catnip plants and aphids were present in the greenhouse. One of the catnip plants developed a mottling and chlorosis that strikingly resembled symptoms of the mosaic disease. Attempts were made to transmit mosaic infection from the catnip to tomato *(Lycopersicon esculentum)* and attempts were made to transmit mosaic from tobacco *(Nicotiana tabacum)* to catnip.

SUMMARY OF TABLE X.

Five tomato plants were inoculated on March 25, with mosaic catnip tissue and five were held as checks. Three of the inoculated plants exhibited mosaic symptoms 16 days following the inoculations while the checks remained healthy. The catnip plant was accidentally killed before further inoculations were made. A number of attempts were made to artificially transmit mosaic infection from tobacco to catnip. Of 34 catnip plants which were inoculated, none were infected.

The characteristic mosaic symptoms of the catnip plant referred to, and the mosaic infection from this plant to three tomato plants indicate that the species *Nepeta cataria* is a host plant of the mosaic disease. The limited data obtained indicate that the mosaic disease is transmissible from this species of the Labiateae to the Solanaceae.

Mosaic of Abutilon theophrasti.

Velvet leaf plants *(Abutilon theophrasti),* were found growing in a cucumber field near Ottumwa, Iowa, in September, 1922, infected with the mosaic disease. The infected plants exhibited a

TABLE X. CROSS INOCULATION TRIALS WITH THE MOSAIC VIRUS FROM *NEPETA CATARIA*

Date of inoculation	Source of virus	Species inoculated	Methods‡	Results			Length of exp. in days
				No. inoculated	Infected	No.† checks	
1922							
3-25	*Nepeta cataria*	*Lycopersicon esculentum*	TF	5	3	10	21
4-20	*Nicotiana tabacum*	*Nepeta cataria*	TF	10	0	10	30
1923							
3- 2	" "	" "	TF	3	0	3	32
4- 9	" "	" "	TF	5	0	5	30
5- 4	" "	" "	TF	6	0	6	40
7-16	" "	" "	TF	10	0	10	25

†None of the checks were infected.
‡TF stands for tissue fragment.

TABLE XI. CROSS INOCULATION TRIALS WITH THE MOSAIC VIRUS FROM *ABUTILON THEOPHRASTI*

Date of inoculation	Source of virus	Species inoculated	Methods‡	Results			Length of exp. in days
				No. inoculated	Infected	No.† checks	
1922							
8-22	*Abutilon theophrasti*	*Abutilon theophrasti*	TF	10	8	10	24
10-10	" "	*Nicotiana tabacum*	TF	5	1	5	55
10-10	" "	*Lycopersicon esculentum*	TF	5	5	5	55
1923							
7-16	*Nicotiana tabacum*	*Abutilon theophrasti*	P	12	0	12	48

†None of the checks were infected.
‡TF stands for tissue fragment and P for puncture.

marked mosaic-like mottling, especially on the younger leaves. Cucumber mosaic was also prevalent in this field.

Tissue inoculations were made in the field from mosaic infected to healthy velvet leaf plants. A number of the infected plants were taken to the Experiment Station where cross inoculations were made under controlled greenhouse conditions to tobacco *(Nicotiana tabacum)*, and tomato *(Lycopersicon esculentum)*.

SUMMARY OF TABLE XI. --

Mosaic velvet leaf tissue was inoculated on August 22, 1922, to 10 healthy plants of this species. The inoculated plants were not caged. Ten velvet leaf plants that were growing within a few feet of the inoculated ones were used as checks. On September 15, when these plants were next observed, 8 of the 10 inoculated plants had developed mosaic symptoms. All checks were healthy.

Five plants each of tobacco and tomato were inoculated with mosaic velvet leaf tissue on October 10, 1922. One of the tobacco plants became infected and exhibited mosaic symptoms after an incubation period of 22 days. The five tomato plants all became infected and exhibited mosaic symptoms from 20 to 24 days after inoculation. An equal number of checks were held both of tobacco and tomato and these remained healthy.

Twelve velvet leaf plants growing in the field were inoculated on July 16, 1923, with tobacco mosaic but infection did not result.

Evidence was obtained that *Abutilon theophrasti* is a host of the mosaic disease. Infections were secured from mosaic plants to healthy plants of this species. Cross infections were successful to tobacco and to tomato, indicating that the mosaic virus is transmissible from this species of the Malvaceae to the Solanaceae.

Mosaic of Euphorbia preslii.

Spurge plants *(Euphorbia preslii)* with a mosaic-like mottling were found in August, 1923, in a cucumber field near Des

Moines. From the appearance of these plants the mosaic disease was suspected. Cucumber mosaic was very severe in the field where the spurge plants were found and in this field were also found mosaic infected milkweed *(Asclepias syriaca)* and two species of mosaic infected Physalis. Some of the affected spurge plants were taken to the Experiment Station and were transplanted to pots. Artificial inoculations were made with mosaic spurge tissue to tobacco *(Nicotiana tabacum)*, *tomato (Lycopersicon esculentum)* and to cucumber *(Cucumis sativus)*.

SUMMARY OF TABLE XII.

Ten tobacco plants were inoculated on August 27, 1923, with mosaic spurge tissue. Inoculum was prepared for five of these by macerating the tissue in an acetone solution and for the other five the inoculum was macerated in water. Infection resulted in both series and four plants developed mosaic symptoms after 15 to 22 days following the inoculations. Ten check plants were held and all remained healthy.

On August 27, 1923, 20 cucumber plants were inoculated with mosaic spurge tissue but infection did not result.

Inoculations were made on September 13, 1923, with mosaic spurge juice to 10 tobacco, 14 tomato and 8 cucumber plants. The only infection resulting was in one of the cucumber plants. An equal number of checks of each of the three species were held and these all remained healthy.

Further inoculations were not made as the spurge plants that were transplanted to the greenhouse died ond no more infected plants could be obtained.

From the symptoms exhibited by the *Euphorbia preslii* plants and from the cross infections that were obtained to tobacco and

TABLE XII. CROSS INOCULATION TRIALS WITH THE MOSAIC VIRUS FROM *EUPHORBIA PRESLII*

Date of inoculation	Source of virus	Species inoculated	Methods‡	Results			Length of exp. in days
				No. inoculated	Infected	No.† checks	
1923	*Euphorbia presslii*						
8-27	" "	*Nicotiana tabacum*	P*	5	3	5	52
8-27	" "	" "	P	5	1	5	52
9-13	" "	" "	P*	5	0	5	59
9-13	" "	" "	P	5	0	5	59
	" "	*Lycopersicon esculentum*					
9-13	" "	" "	P*	7	0	7	59
9-13		" "	P	7	0	7	59
8-27	" "	*Cucumis sativus*	P*	11	0	5	52
8-27	" "	" "	P	9	0	5	52
9-13	" "	" "	P*	4	1	4	59
9-13		" "	P	4	0	4	59

*Inoculum macerated in acetone solution.
†None of the checks were infected.
‡P stands for puncture.

cucumber, evidence was obtained that these plants were infected with the mosaic disease. These cross infections indicate that the mosaic virus is transmissible from the Euphorbiaceae to the Solanaceae and Cucurbitaceae.

Mosaic of Aquilegia spp.

During the summer of 1923 plants of the two species of columbine, *Aquilegia canadensis* and *Aquilegia coerulea,* were found at Ames affected with a disease which has proven to be mosaic. The leaves of affected plants exhibited a characteristic mosaic mottling, this symptom being especially noticeable on the younger leaves. Inoculations were made from mosaic to healthy columbine and also from the mosaic plants to tobacco *(Nicotiana tabacum)* and to tomato *(Lycopersicon esculentum).* The results obtained from these inoculation experiments are given in table XIII.

SUMMARY OF TABLE XIII.

Two attempts were made to transmit mosaic thru the medium of aphids (species undetermined) from *Aquilegia canadensis* to *A. coerulea.* Six of the eight plants that were infested with the aphids developed mosaic symptoms after a period of from 17 to 21 days. Thirty check plants were held for these two experiments and all remained healthy.

Ten tobacco plants were inoculated on September 14, 1923, with juice from mosaic plants of *A. canadensis.* Six of these became infected and exhibited mosaic symptoms from 15 to 22 days following the inoculations. An equal number of checks were held and all remained healthy.

On September 14, 1923, fourteen tomato plants were inocu-

TABLE XIII. CROSS INOCULATION TRIALS WITH THE MOSAIC VIRUS FROM *AQUILEGIA SPP.*

Date of inoculation	Source of virus	Species inoculated	Methods‡	Results			Length of exp. in days
				No. inoculated	Infected	No.† checks	
1923							
9-15	*Aquilegia canadensis*	*Aquilegia coerulea*	A**	3	2	25	40
10-13	" "	" "	A**	5	4	5	33
9-14	" "	*Nicotiana tabacum*	P*	5	2	5	50
9-14	" "	" "	P	5	4	5	50
9-14		*Lycopersicon*					
9-14	" "	*esculentum*	P*	7	0	7	50
	" "	" "	P	7	0	7	50
10-12	*Aquilegia coerulea*	*Nicotiana tabacum*	P*	5	0	5	52
10-12	" "	" "	P	5	3	5	52
12-12	" "	" "	P	5	3	5	29

†None of the checks were infected.
‡A stands for aphids and P for puncture.
*Inoculum macerated in acetone solution.
**Species undetermined.

lated with mosaic from *A. canadensis,* but infections did not result.

Ten tobacco plants were inoculated on October 12, 1923, with mosaic juice from *A. coerulea.* Five plants were inoculated with the columbine tissue macerated in water, of which three plants became infected within 18 days following the inoculations. The remaining five plants were inoculated with the mosaic columbine tissue macerated in acetone, but these did not become infected. Ten tobacco plants were held as checks and all remained healthy.

Five tobacco plants were inoculated on December 12, 1923, with mosaic juice from *A. coerulea.* Three of these plants became infected and exhibited mosaic symptoms in 15 days. The five check plants remained healthy.

Evidence was obtained that *Aquilegia canadensis* and *Aquilegia coerulea* are susceptible to the mosaic disease. Mosaic infection was transmitted thru the medium of aphids from *A. canadensis* to six plants of *A. coerulea.* Artificial inoculations were made to 25 tobacco plants of which 12 became infected. These data give evidence that the mosaic disease is transmissible from species of Ranunculaceae to Solanaceae.

MOSAIC TRANSMISSION BETWEEN NICOTIANA TABACUM AND NICOTIANA GLUTINOSA.

Allard (3) has reported a specific mosaic disease of *Nicotiana glutinosa** distinct from the mosaic disease of *Nicotiana tabacum.* Successful mosaic infection was neither obtained from *N. tabacum* to *N. glutinosa,* nor from *N. glutinosa* to *N. tabacum. Datura stramonium* was the only species of the Solanaceae that was infected with mosaic from both *Nicotiana glutinosa* and *N. tabacum.* The failure to cross infect mosaic from one of these species of Nicotiana to the other was the basis for Allard's conclusion that each species is subject to a distinct specific mosaic disease.

Allard recorded no attempts to transmit mosaic to *N. tabacum* from *Datura stramonium* plants that had been infected with mosaic from *Nicotiana glutinosa.* The possibility is suggested that *Datura stramonium* may serve as an intermediate host for the successful transmission of mosaic between *Nicotiana glutinosa* and *N. tabacum.* Plants of these three species were propagated in order to secure information on this question; direct mosaic inoculations were made between the species *N. tabacum* and *N. glutinosa.*

*Allard's investigations were made with *Nicotiana glutinosa* instead of with *Nicotiana viscosa* as published according to a letter from Mr. Allard to the writer, containing the following statement: "If you have in mind the species of tobacco with which I carried on experiments with the mosaic disease of tobacco under the name of *Nicotiana viscosa,* I may state that the proper name of the species is *Nicotiana glutinosa* * * *"

TABLE XIV. MOSAIC CROSS INOCULATIONS BETWEEN *NICOTIANA TABACUM* AND *NICOTIANA GLUTINOSA*

Date of inoculation	Source of virus	Species inoculated	Methods‡	Results			Length of exp. in days
				No. inoculated	Infected	No.† checks	
1923							
3-13	*Nicotiana tabacum*	*Datura stramonium*	TF	3	3	3	19
4-15	*Datura stramonium*	*Nicotiana glutinosa*	TF	7	4	10	56
6-15	*Nicotiana glutinosa*	*Datura stramonium*	P*	6	4	6	15
7- 3	*Datura stramonium*	*Nicotiana tabacum*	TF	5	5	5	33
6-13	*Nicotiana glutinosa*	" "	P*	5	0	5	41
9-14	" "	" "	P*	5	5	5	24
9-14	" "	" "	P	5	5	5	24
6-13		*Lycopersicon esculentum*	P*	5	3	5	41
9-14	" "	" "	P*	7	7	7	24
9-14	" "	" "	P	7	6	7	24
7-16	*Nicotiana tabacum*	*Nicotiana glutinosa*	P	5	4	9	31
10-13	" "	" "	P	5	3	5	33

†None of the checks were infected excepting one of the nine *N. glutinosa* plants inoculated on July 16, 1923.
*Inoculum macerated in acetone solution.
‡TF stands for tissue fragment and P for puncture.

SUMMARY OF TABLE XIV.

March 13, 1923, three *Datura stramonium* plants were inoculated with mosaic from *Nicotiana tabacum* and all of these developed the mosaic disease. Leaf tissue of these mosaic *Datura stramonium* plants was inoculated on April 15, 1923, to seven *Nicotiana glutinosa* plants. Mosaic developed in four of these. In order to carry the infection back to *N. tabacum,* six *Datura stramonium* plants were inoculated on June 15, 1923, with mosaic juice from *Nicotiana glutinosa.* Four of the *Datura stramonium* plants developed mosaic infection. From these mosaic *D. stramonium* plants inoculations were made on July 3, 1923, to five *Nicotiana tabacum* plants, all of which became infected. The mosaic virus had in this series of inoculations been transmitted from *N. tabacum* to *N. glutinosa* and back again to *N. tabacum* by the use of *Datura stramonium* as an intermediate host.

A number of attempts were made to transmit mosaic infection directly from *Nicotiana tabacum* to *N. glutinosa* and vice versa. Inoculations of mosaic from *N. glutinosa* were also made to tomato. There were 10 tobacco and 14 tomato plants inoculated on September 14, 1923, with mosaic *N. glutinosa* tissue. Five of the tobacco and seven of the tomato plants were inoculated with the mosaic *N. glutinosa* leaf tissue macerated in a solution of acetone and the remaining tobacco and tomato plants were inoculated with the mosaic leaf tissue macerated in water. An equal number of check plants were held, all of which remained healthy. The 10 tobacco plants and 13 of the 14 tomato plants inoculated became infected.

Five tobacco and five tomato plants were inoculated on June 13, 1923, with mosaic *N. glutinosa* leaves macerated in an acetone solution. Three of the tomato plants developed infection. Five tobacco and five tomato plants were held as checks and all remained healthy.

Two attempts were made to transmit mosaic infection directly from *N. tabacum* to *N. glutinosa*. Five *N. glutinosa* plants growing in the field, were inoculated on July 15, 1923, with mosaic *N. tabacum* leaf tissue that had been macerated in acetone solution. These plants were not caged. Nine plants were held as checks. Of the five inoculated plants four developed infection. The check plant adjacent to the five inoculated plants also developed the mosaic disease.

Five *N. glusinosa* plants growing in the greenhouse were inoculated on October 13, 1923, with mosaic *N. tabacum* tissue that had been macerated in water. Five plants were held as checks, all of which remained healthy. Three of the five inoculated plants developed the mosaic disease.

The results obtained in attempts to cross inoculate mosaic from *N. tabacum* to *N. glusinosa* and vice versa indicate that these species are susceptible to the same specific mosaic virus. Mosaic cross infection was obtained between these species by direct artificial inoculation and by the use of *Datura stramonium* as an intermediate host.

THE ROLE OF INSECTS IN MOSAIC TRANSMISSION.

Allard (1) in 1912 presented evidence showing that aphids play an important role in the dissemination of the mosaic disease. Later Doolittle (24) found that the leaf-eating beetles, *Diabrotica vittata* and *D. duodecimpunctata* are capable of transmitting the mosaic virus. Some additional evidence has been secured concerning the ability of certain insects to serve as mosaic vectors. These results are summarized in table XV.

SUMMARY OF TABLE XV.

In an earlier chapter it was noted that mealy bugs *(Pseudococcus maritimus* Ehr.) served as transmitting agents of the mosaic virus. Infection was transmitted by these insects from crookneck squash *(Cucurbita pepo* var. *condensa),* to cow pea *(Vigna sinensis),* tomato *(Lycopersicon esculentum),* and tobacco *(Nicotiana tabacum);* and from egg plant *(Solanum melongena)* to cow pea.

In addition, mealy bugs transmitted mosaic infection from cow peas to soy beans *(Soja max)* and from soy beans to cow peas. Mealy bugs from mosaic infected cow peas were transferred on May 10, 1922, to 17 soybean plants. The pots in which these plants were growing were held in an insect proof cage. All of

TABLE XV. MOSAIC CROSS-INFECTIONS OBTAINED THRU THE MEDIUM OF INSECTS

Date of inoculation	Source of virus	Species inoculated	Methods‡	Results			Length of exp. in days
				No. inoculated	Infected	No. checks†	
1922							
3- 1	*Vigna sinensis*	*Vigna sinensis*	AS	17	17	150	31
3-14	" "	" "	AS	38	38	32	14
3-30	" "	" "	AS	26	21	45	16
5-18	*Solanum tuberosum*	" "	AS	38	38	60	21
1923							
6-14	*Heliopsis scabra*	*Heliopsis scabra*	AS	3	3	20	18
9-15	*Aquilegia canadensis*	*Aquilegia coerulea*	AS	3	2	25	30
10-13	" "	" "	AS	5	4	5	33
	Saccharum						
9- 2	*officinarum*	*Zea mays*	AM	19	9	0	23
9-19	" "	*Achyrodes aureum*	AM	6	1	20	22
11-15	*Achyrodes aureum*	*Zea mays* (yellow dent)	AM	7	3	14	18
11-15	*Zea mays*	*Zea mays* (sweet corn)	AM	7	2	15	18
11-30	*Cucumis sativus*	*Vigna sinensis*	AG	6	3	15	17
12-21	*Apium graveolens*	*Cucumis sativus*	AG	2	2	2	14
12-26	" "	" "	AG	13	10	10	17
1924							
1-15	" "	" "	AG	10	8	10	14
1922		*Lycopersicon*					
4-11	*Cucurbita pepo*	*esculentum*	PM	5	1	5	34
4-11	"	*Nicotiana tabacum*	PM	5	2	5	34
3-30	"	*Vigna sinensis*	PM	16	16	11	15
4- 8	" "	" "	PM	32	20	21	15
5-8	*Solanum melongena*	" "	PM	36	28	60	21
5- 6	" "	" "	PM	14	8	16	30
6- 6	*Soja max*	" "	PM	13	13	13	19
5-10	*Vigna sinensis*	*Soja max*	PM	17	7	18	22
8—	*Nicotiana tabacum*	*Nicotiana tabacum*	PS	8	7	80	30

†None of the checks were infected.
‡AS stands for *Aphis spp.*, AM for *Aphis maidis*, AG for *Aphis gossyppi*, PM for *Pseudococcus maritimus* and PS for *Protoparce sexta.*

the 18 plants which were held as controls remained healthy. Seven of the soybean plants infested with the mealy bugs developed mosaic infection.

On June 6, 1922, mealy bugs from mosaic infected soybean plants were transferred to 13 healthy cowpea seedlings and the pot was placed in an insect proof cage. One hundred percent infection resulted, while an equal number of plants held as checks remained healthy.

As indicated in table XV, aphids (species undetermined) transmitted the mosaic virus from infected cow pea and potato to cowpea plants. Thru the medium *Aphis gossypii* from mosaic infected cucumber plants, mosaic infection was transmitted to cow pea plants, and this aphid has transmitted mosaic from infected celery to cucumbers. *Heliopsis scabra* plants were infected with mosaic by using aphids (species undetermined) from mosaic infected *H. scabra* plants. *Aquilegia coerulea* was likewise infected with the virus from mosaic infected *A. canadensis plants.*

Aphis maidis transmitted mosaic infection from sugar cane *(Saccharum officinarum)* to corn *(Zea mays* var. Yellow Dent) and to *Achyrodes aureum;* from mosaic infected Yellow Dent corn to healthy sweet corn; and from *Achyrodes aureum* to Yellow Dent corn.

In other experiments the tobacco hornworm *(Protoparce sexta* Johan.) served as an agent for mosaic transmission. In August, 1922, eight of these worms were placed on a caged mosaic tobacco plant and were allowed to feed for a day. At the end of this time each of these worms was transferred to a healthy tobacco plant. These plants were growing in the field and an insect proof cage was placed over each of the eight plants thus infested. The worms were allowed to feed on the tobacco plants for a day. Care was taken that accidental infection did not occur in the process of placing and removing them. In transferring the worms to the tobacco plants they were placed on the ground near their host without the operator coming in contact with the plants. Tweezers were used in removing them and the plant was not touched. Seven of the eight plants became infected. In the remainder of the two rows of approximately 80 tobacco plants which served as checks, no case of mosaic appeared at any time during or following this experiment.

Table XV summarizes the mosaic cross infections that were obtained thru the medium of insects. Most of these data have been included in previous tables where insect and artificial inoculations were presented together.

PATHOLOGICAL EFFECT OF MOSAIC

The symptoms of mosaic in general may be mottling; shoestring or other malformation of the leaves; malformation or abnormal pigmentation of the flowers (Allard, 4); malformation, abnormal pigmentation and reduced yield of the fruit (Gardner and Kendrick, 31); and general stunting. In certain species a high percentage of sterility of seeds is produced (Dickson and McRostie, 20). An affected plant may exhibit any one or various of these symptoms in combination.

No one working with the mosaic disease can fail to notice the wide variation of symptoms in different infected plants or on different leaves of the same plant. Variable symptoms on distinct parts of the same plant are evidently the result of internal responses to mosaic infection.

Plants of a given species growing under different environmental conditions may exhibit distinct symptoms. The catacoralla of the flowers of *Nicotiana tabacum* described by Allard (4) have not been found in Iowa, altho hundreds of mosaic tobacco flowers were examined. Dixon (21) of Canada, and Gard-

ner and Kendrick (31) picture and describe mottling of mosaic tomato fruit and report it as a common symptom. A faint mottling of tomato fruit was found in Iowa only once and this symptom is very unusual under Iowa conditions. MacMillan (45) reported that in potato the mosaic symptoms are entirely masked at altitudes above 8,000 feet, while in lower alitudes mottling of the leaves is evident. It has been observed in potatoes affected with the mosaic disease that the intensity of sunlight results in different degrees of mottling of the leaves. In the intense light of summer mottling is more pronounced on shaded than on unshaded plants. The mosaic symptom-complex exhibited in different plants of the same variety may be due in part to internal responses; again, these symptoms may be due to both the internal responses and the response to environmental conditions.

The mosaic symptoms exhibited by any given species are in general unlike those produced on other species. This variability of symptoms in different species is often due to morphological differences. The mosaic mottling of sugar cane leaves is a longitudinal streaking which differs from the characteristic mottling of mosaic tobacco leaves. Other factors besides morphology influence the characteristic symptoms for a species. A difference in the physiological response to the mosaic virus is suggested for species that exhibit mosaic mottling and for species that, altho susceptible to the virus, do not exhibit mottling symptoms.

MASKING OF SYMPTOMS.

Mosaic is not recognizable in all infected plants by the exhibition of mottling symptoms. Plants belonging to certain species may be affected with mosaic and exhibit no evident symptoms of abnormal pigmentation. Mosaic infected plants that exhibit no abnormal chlorosis are said to carry the disease in a masked condition.

Allard (4) noticed that *Nicotiana glauca* plants after becoming infected soon lost the symptoms of mottling even tho the mosaic causal agent was still highly virulent as was proved by inoculation experiments. Melhus (48) found that mosaic infected potato *(Solanum tuberosum),* the parent plants of which had been grown in Maine where these had produced definite mosaic mottling, failed to produce plants with evident mottling when grown under Iowa conditions. Melhus has shown further that infected egg plant *(Solanum melongena)* seedlings exhibit mosaic symptoms, but that these symptoms disappear when the seedling stage is passed.

Not only may mosaic infected plants lose the evident mottling symptoms that are produced due to this disease, but certain species never produce evident mottling symptoms. Nishimura (50) has reported this condition in the species *Physalis alkekengi* and

an analogous case was reported by Bauer (7) in the infectious chlorosis of the Malvaceae.

The sudden disappearance of mosaic symptoms in plants where such symptoms had been evident has led to a number of investigations to determine if this disappearance was due to the plant's recovery from the disease. In most of these investigations (Dickson, 21), it was found that the mosaic virus within the plant was still virulent. Brierly (11) concluded in the case of a tomato from which mosaic symptoms had disappeared, that recovery had taken place. This conclusion was based on negative results in one inoculation trial.

Brandes reported that in the case of corn, crab grass, sugar cane, sorghum, and fox tail several cases of apparent recovery were noticed in that new unmottled growth was produced on plants that previously had produced mosaic mottling. Lyon (44) also reported recovery of sugar cane.

Evidence was secured in these investigations concerning the complete and permanent masking of symptoms in certain species of mosaic susceptible plants and concerning the masking of mosaic symptoms under particular environmental conditions.

Plants of *Physalis francheti* (a species closely related to *P. alkekengi,* but being an annual and having larger fruiting calyces) were in these investigations found to be susceptible to mosaic infection altho mottling symptoms were entirely masked. Five of these plants were inoculated with mosaic infected tobacco tissue, but none developed mosaic symptoms. Inoculations were made to tobacco with young leaf tissue that developed after the Physalis plants were inoculated. Infection was obtained in the tobacco, indicating that the *Physalis francheti* plants carried the mosaic disease in a masked condition.

Celery *(Apium graveolens)* was found in August, 1923, infected with the mosaic disease. Many of the infected plants exhibited no mottling; the only recognizable symptoms in many plants was the presence of filiform or shoe-string leaves. These celery plants were proved to be infected by inoculation tests (table VI). Four celery plants whose only evident mosaic symptom was the filiform leaves were transplanted to pots in the greenhouse. Leaves were produced by those plants in October that exhibited mosaic mottling but later in the year this mottling again disappeared. Thruout the winter the only recognizable mosaic symptom on these plants was the production of filiform leaves. It appears that the mosaic celery plants continued to exhibit the filiform leaf symptom under environmental factors that cause leaf mottling to disappear.

Certain plants that under some environmental conditions do not exhibit mottling symptoms may develop such symptoms when grown under other environmental conditions. A Mexican

variety of beans, known as Berrendo, was reported by Barss (6) to be susceptible to the mosaic disease but produced no evident mottling symptoms under western Oregon conditions. Berrendo bean seed was obtained from Oregon and was grown during the summer of 1923 in the open at Ames. In order to study the reaction of this variety to mosaic infection, these beans were colonized with aphids (species undetermined) from other varieties of mosaic infected beans. It was found that under Iowa conditions the Berrendo bean produces mosaic mottling, altho this mottling was not as striking as is the case for certain other varieties of beans. Seeds collected from mosaic Berrendo bean plants were planted in the greenhouse and of the 12 plants grown, 4 exhibited the mosaic mottling symptoms.

Mosaic symptoms in egg plant *(Solanum melongena),* as reported by Melhus, are usually not evident in infected plants that are past the seedling stage. An exception was observed in the case of a very vigorously growing egg plant that exhibited decided mottling at the time the plant had produced a mature fruit. This plant was growing in a warm greenhouse under conditions where a rank succulent growth was produced.

Masking of mosaic mottling may or may not be influenced by environmental conditions. In such plants as *Physalis alkekengi* mosaic mottling, so far as is known, does not develop under any environmental condition. The plant itself appears to be resistant to the disturbance that results in abnormal chlorophyll production. In such plants as tobacco, however, one of the effects of the mosaic causal agent is the unbalancing of the normal production of the four unit pigments of chlorphyll in distinct areas and an abnormally pigmented leaf is produced. In species where mosaic mottling is produced, environment is an important factor in influencing the degree of this abnormality. The mottled or masked condition of celery, Berrendo bean, and egg plant are illustrations of this fact.

THE EFFECT OF PHYSICAL STIMULI ON MOSAIC SYMPTOMS.

Mosaic symptoms are the evident response of a plant to infection. Why the mottling symptoms should in certain cases become masked is a question of considerable interest.

Light plays an important role in the development of chlorosis in plants infected with mosaic. Lodewitz (43), Chapman (13) and Dixon (21) found that the red rays of the spectrum do not influence mottling or masking of symptoms in affected plants. The blue rays, however, have a decided influence on mottling. Mosaic plants grown in red light retained their mottling but when grown in blue light the mottling disappeared. The mosaic virus was not destroyed in plants where mosaic symptoms were

masked due to blue light as was shown both from inoculation experiments (Chapman, 13) and from the fact that upon removing the plants to sunlight newly produced leaves developed mosaic mottling. From these results it would appear that the variability of mosaic symptoms to red or blue light is no index of an effect on the mosaic causal agent itself. It appears, rather, that the variation of symptoms produced by red or blue light indicates differences in the host's response to the infection.

Evidence has been obtained by Doolittle (25), Johnson (40) (41) and Dixon (22) that temperature plays an important role in determining the degree to which a mosaic plant exhibits evident mottling symptoms.

Investigations have been made by Johnson concerning the response of the mosaic virus itself to temperature. He (40) states that for the causal agent of the mosaic disease of tobacco "* * * * the optimum temperature for the activity of the virus appears to be between 28° and 30° C. and the maximum temperature is close to 36° C." In a later publication (41) similar investigations were made with mosaic potatoes, tomatoes, clover, soy bean and pea beans. The optimum for potato mosaic was found to be between 14° and 18° C., while the maximum was 24° to 25° C. Optimum temperatures for mosaic development in the other hosts tested were found to vary for each of these hosts.

The question arises whether the temperatures found by Johnson as optimum were not the optimum temperatures for a vigorous vegetative development of the hosts with which the tests were made.

Two experiments were made to secure information concerning the comparative importance of temperature and vigor of growth to mosaic infection as expressed in the length of the mosaic incubation periods following inoculation. Tomato plants were used for these experiments that up to the date the experiments were begun had been grown on the same bench. The inoculations were made with mosaic tomato tissue.

The tomato plants were divided into two groups in both experiments. One-half of the plants were grown in a cold and the other half were grown in a hot greenhouse for a period of 14 days previous to the date of inoculation. On the date of inoculation one-half of the plants from the cold house were transferred to the hot house and one-half of the plants from the hot house were transferred to the cold house. The four series were kept in these houses during the entire incubation period and were designated as follows:

Series A, kept in hot house during entire experiment.
Series B, kept in cold house during entire experiment.
Series C, moved from cold to hot house on inoculation date.
Series D, moved from hot to cold house on inoculation date.

EXPERIMENT NO. 1.

The first of these experiments was made in the spring of 1922, using tomato plants that were approximately six inches tall.

Thermograph records were taken in both greenhouses. During the mosaic incubation period the night and day temperatures in the hot greenhouse averaged about 25° and 27° C., respectively. These temperatures averaged about 12° and 18° C., respectively, for the cold greenhouse.

The seven plants of Series A that were kept in the hot house during the entire period produced a continuous vigorous growth. The nine plants of Series C transferred from the cold to the hot house began growing vigorously following the date of the inoculations. The nine plants of Series B, which were kept in the cold greenhouse during the entire experiment, had on the date of inoculation recovered from the shock of the changed environment and were producing a slow stocky growth. The six plants that were transferred from the hot to the cold house (Series D) received a very apparent check and did not make a noticeable growth during the first 14 days following the inoculations.

The results of the experiment showed that the plants in the series which were in the best condition for vigorous growth exhibited mosaic symptoms in a shorter time than the plants in the series that were checked due to the sudden change of environmental conditions. Although Series A and C were both growing in the hot house during the entire incubation period, this period was approximately five days shorter for the plants of Series A than of Series C. Similar results were obtained for the series growing in the cold greenhouse where the incubation period length was approximately seven days shorter for Series B than for Series D, altho both series were growing under the same environmental condition.

EXPERIMENT NO. 2.

In the spring of 1924 a second attempt was made to secure data concerning the comparative importance of the temperature and vigor of growth to the length of the mosaic incubation period. Twenty tomato plants were placed on March 21 in each of the two greenhouses utilized for the experiment in 1922 and, as in the previous experiment, the plants were held in these houses for 14 days previous to the date of inoculation.

Thermograph records were taken of the temperatures of both houses. During the incubation period the night and day temperatures of the hot greenhouse averaged about 24° and 27° C., respectively; these temperatures averaged about 10° and 17° C., respectively, for the cold greenhouse.

March 21, when the tomato plants were placed in the hot and the cold houses, respectively, the plants, which were in five-inch

pots, were all approximately 18 inches tall and were growing vigorously. In the 14 days intervening between the date the plants were placed in the respective houses and April 14, the date of inoculation, the plants that were kept in the hot house (Series A and D) had stopped growing vigorously due to their roots becoming bound in the pots. The plants in the cold house made but a very slow growth during the weeks preceding the inoculation and were at the date of inoculation not yet incapable of rapid growth due to their soil substratum.

Inoculations were made on April 14 and the plants were divided into Series A, B, C, D; each series containing 10 plants. The series were held in the hot and cold greenhouses in the same order as were the series in Experiment No. 1.

The results obtained are in agreement with those obtained in Experiment No. 1; they indicate that the vigor of growth of inoculated plants is of great importance in determining the length of the mosaic incubation period. The plants of Series A and D did not grow vigorously at any time after the inoculation date. The plants of both these series, being root bound, were unable to produce a vigorus growth and the plants of Series D were furthermore shocked by the change from the hot to the cold environment. The plants of the series kept in the cool greenhouse during the 14 day period previous to the date of inoculation (Series B and C) were not root bound and were capable of vigorous growth following the inoculation date. Of the series held in the hot house following inoculation, the plants of Series C developed mosaic symptoms after an incubation period averaging six days less than did the plants of Series A. In the series held in the cold house following inoculation, the plants of Series B developed symptoms after a period averaging 11 days less than did the plants of Series D.

The available soil food supply was a greater limiting factor in influencing vigor of growth in the plants of these series than was the temperature. From the fact that the shortest mosaic incubation period resulted in the series that, following the inoculations, produced the more vigorous vegetative growth, it appears that growth, vigor determinated the length of the mosaic incubation period in these series.

An experiment was begun March 21, 1924, to secure data concerning the effects of stunting on the length of the mosaic incubation period. Twenty tomato plants were used in this experiment. Ten of the 20 plants were supplied with a sufficient supply of water for normal growth thruout the experiment. For a period of 14 days the remaining 10 plants were supplied with just enough water to keep them from dying. At the end of the 14 days all plants were inoculated with mosaic tomato tissue. The plants used

in this experiment were approximately 16 inches tall on March 21 and those that had been well watered during the first two weeks had at the end of this period become root bound and made very little growth following the date of inoculation. The 10 plants that were kept dry for the 14 days previous to inoculation were subsequently well watered and were soon growing vigorously. During the entire mosaic incubation period both series were growing on the same bench under the same conditions of heat, light and moisture supply.

All of the plants of the series that were kept dry for the 14 days previous to inoculation exhibited mosaic symptoms before the plants of the other series, the average difference in time being six days. It is suggested that the difference in the length of the mosaic incubation period was because following inoculation the plants of one series were growing rapidly while those of the other were not. These results and numerous observations suggest that plants growing in optimum environmental conditions for vegetative growth will exhibit symptoms after a shorter incubation period following mosaic infection than will plants that are not making a vigorous growth.

EARLY SYMPTOMS IN MOSAIC INFECTED PLANTS.

Definite mottling or malformation is not in all cases the first recognizable mosaic symptom of an infected plant. Among numerous tobacco plants that were inoculated during the winter months, it was possible to identify infected plants previous to the time when definite mottling or malformation was exhibited. Normal young tobacco leaves are uniform in color and the veins may be seen as distinct lines. This is especially true when viewed by transmitted light. In infected leaves showing mosaic symptoms before mottling was apparent, the definite pattern of the veins was not so clear cut as in healthy plants owing to a bleached appearance of the leaf blade in a narrow area paralleling the veinlets. The veins became indistinct owing to the more gradual diffusion of light to green from the lighter colored cells of the veins to the green chlorophyll-bearing cells on either side. Leaves that were well formed at the time mosaic infection occurred did not develop mottling symptoms. Following infection, mottling appeared on young leaves that were in the meristematic stage at the time of infection and such mottling sometimes developed on all leaves produced subsequently. The early mosaic symptoms that were observed were found on leaves that were in an intermediate condition between the more mature and the juvenile leaves. Evidently the leaves had passed the growth period where the effect of the mosaic causal agent could influence abnormalities in tissue differentiation and pigment production as much as it does in younger leaves. This effect was, however, still present

to less degree as evidenced by the abnormalities in the pigmentation of chlorophyll bearing cells in the vicinity of the veins. Leaves producing these early symptoms were never observed to produce mosaic mottling later on. It was observed that after a period of time these leaves lost all mosaic symptoms so that at maturity they were similar in appearance to leaves that were mature at the date the plant became infected.

Infected cucumber plants were observed with early mosaic symptoms similar to those described for tobacco.

Carsner (12) (discussion concerning abstract) described the occurrence of early symptoms in sugar-beet leaves affected with curly-top that are very similar to the early symptoms on tobacco leaves affected with mosaic.

The production of these early mosaic symptoms makes it possible to recognize infected plants a number of days sooner than is otherwise possible. In every instance where such early mosaic symptoms occurred, the plant exhibiting these later developed leaves that exhibited definite mosaic mottling.

EFFECT OF THE MOSAIC DISEASE ON CHLOROPHYLL

From only a superficial observation of mosaic tobacco plants, as well as other hosts exhibiting the mosaic symptom of mottling, it is evident that an effect of the mosaic disease is the unbalancing of the chlorophyll content of such plants. As compared with leaves of healthy plants the leaves of mosaic plants are divided into irregular areas, some of which are a lighter green or yellowish color, while the contrasting areas are a darker green than normal. No quantitative determination of the nature of the abnormal chlorotic condition of mosaic diseased tissue has been found recorded in the literature.

Wilstatter and his associates in their classic investigations on plant pigments have succeeded in determining the chemical nature of chlorophyll. Their investigations have shown that chlorophyll is composed of four unit pigments of which two, Phytochlorin (Chlorophyll A) and Phytorhodin (Chlorophyll B) are green. The other two, carotin and xanthophyll, are yellow. Wilstatter and Stoll (64) have outlined a method by which quantitative determinations of the chlorophyll pigments of leaves may be made.

The separation of the four unit pigments by the method developed by Willstatter and his associates is based on the difference in solubility of these pigments to various solvents. Phytochlorin is soluble in a three percent solution of hydrochloric acid while Phytorhodin requires a higher concentration and after fractioning off Phytochlorin with the three percent hyprochloric acid, Phytorhodin is extracted with a 12 percent solution of this

acid. Xanthophyll is soluble while carotin is not soluble in methyl alcohol. Carotin and xanthophyll are both soluble in petrolic ether. The xanthophyll is fractioned from the carotin with methyl alcohol, after which the carotin is extracted with petrolic ether.

EXPERIMENTAL DATA.

For the purpose of determining the pathological effect of the mosaic disease on chlorophyll production, the chlorophyll content of mosaic leaves was extracted and the four pigments were quantitatively separated. A quantitative comparison of these pigments was made with the chlorophyll content from healthy leaves. The data obtained indicate the percent of the unit pigments that were present in mosaic leaves when compared to the normal pigment content of healthy leaves. The methods outlined by Wilsatter and Stoll (64) were followed.

Tobacco *(Nicotiana tabacum)* and tomato *(Lycopersicon esculentum)* were used in these investigations. The chlorophyll determinations of mosaic infected tobacco were made separately for the light and dark green areas. The samples of light green and dark green tissues were obtained from the same leaves.

No separation of dark and light green areas was made in the tomato leaves used in the chlorophyll determinations. The mottling of tomato leaves is in smaller areas and great difficulty would be encountered in any attempt to separate these within the time necessary to secure an equal gram weight of leaves for the comparative tests. The mosaic tomato leaves used were of a very chlorotic nature, that is, the leaves as a whole were comparable in appearance to the light green areas of the tobacco leaves used. The determinations with tomato leaves were made in May, 1922, and the tobacco leaf determinations were made in January, 1923. In selecting leaves for the chlorophyll determinations, care was taken to use leaves from mosaic and healthy plants that were comparable with respect to age, vigor of growth and position on the plant. Likewise plants were selected that were growing under similar environmental conditions.

As the purpose of this investigation was to ascertain the nature of the abnormal chlorophyll content of mosaic leaves as compared to that of healthy leaves, no attempt was made to determine the dry weight of the components per gram of leaf tissue. Having quantitatively extracted and separated the four unit pigments from like quantities of leaves of both mosaic and healthy plants, the amount of pigment present in the mosaic and the healthy leaves was compared colorimetrically. A Bausch and Lomb Duboscque colorimeter was used for this purpose. The amount of pigment present in the healthy leaves was used as a standard and was given a value of 100. The comparative values

TABLE XVI. CHLOROPHYLL COMPONENTS IN HEALTHY AND MOSAIC LEAVES

Component	Tomato		Tobacco		
	Healthy leaves	Mosaic leaves	Healthy leaves	Mosaic leaves Dark green areas	Mosaic leaves Light green areas
Phytochlorin	100	55	100	130	64
Phytorhodin	100	70	100	169	94
Xanthophyll	100	64	100	79	68
Carotin	100	195	100	237	196

of the unit pigments of healthy and mosaic leaves are listed in table XVI.

The data in table XVI shows that the dark green areas of tobacco leaves contained a larger amount of the green pigments than did the light green areas. The dark green areas of mosaic tobacco leaves contained more phytochlorin and phytorhodin than did healthy leaves; the light green areas contained less phytochlorin and phytorhodin than did healthy leaves. Mosaic leaves, both in the light green and in the dark green areas, contained less xanthophyll than did healthy leaves. Mosaic leaves, both in tomato and tobacco contained approximately double the normal amount of carotin. While the quantity of green components varied in the light green and the dark green areas of mosaic leaves, the yellow components were found present in comparatively similar quantities in both the light green and the dark green areas.

DISCUSSION

The evidence presented indicates that the mosaic virus is transmissible among species that are widely separated taxonomically. In the investigations herein reported, mosaic transmission was obtained among species belonging to 15 families and 11 orders. The species concerned in these inter-family and inter-order cross inoculations are shown graphically in figs. 1 and 2.

It might be argued that the wide range of mosaic infection obtained was possibly due to the utilization of a specific cosmopolitan mosaic virus. The infected plants used as sources of mosaic inoculation were, however, obtained from widely separated localities which greatly reduces the likelihood that such was the case. Infected bean plants used in transmitting the mosaic virus to Solonaceae (table I) were infected thru seed transmission, this seed having been secured from New York*; infected sugar cane plants, from which tissue was used as mosaic inoculum (table V), were obtained from Louisiana**; infected raspberries from which mosaic transmission was secured to tobacco (table VII) were obtained from Canada***; mosaic

*From Dr. Donald Reddick, Cornell University.
**Thru Dr. C. W. Edgerton, La. Agr. Exp. Sta.
***From Prof. J. F. Hockey, Dominion Exp. Farms, St. Cathraines, Ont., Canada.

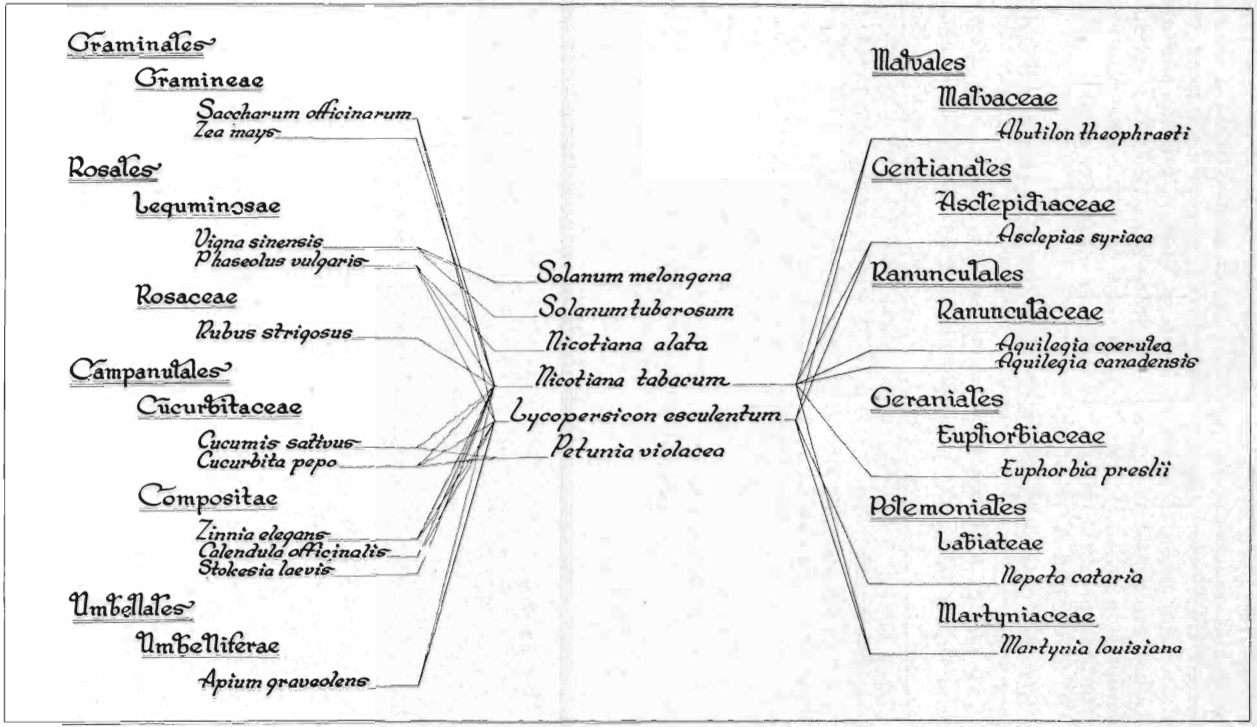

Fig. 1. Mosaic transmission to Solanaceae.

infected Asclepias and Abutilon plants (tables VIII and XI) were found at points several miles apart near Ottumwa, Iowa; infected Euphorbia plants (table XII) were found near Des Moines; and mosaic columbine, celery, catnip, zinnia, and calendula, were found at Ames. If it were granted that a specific cosmopolitan mosaic virus was utilized in these investigations it would appear that this cosmopolitan mosaic is present in all of the above named localities.

Successful artificial cross inoculations to tobacco from species belonging to distinct families and orders were more difficult to obtain and the incubation period was usually longer than were inoculations from infected tobacco or tomato. Once

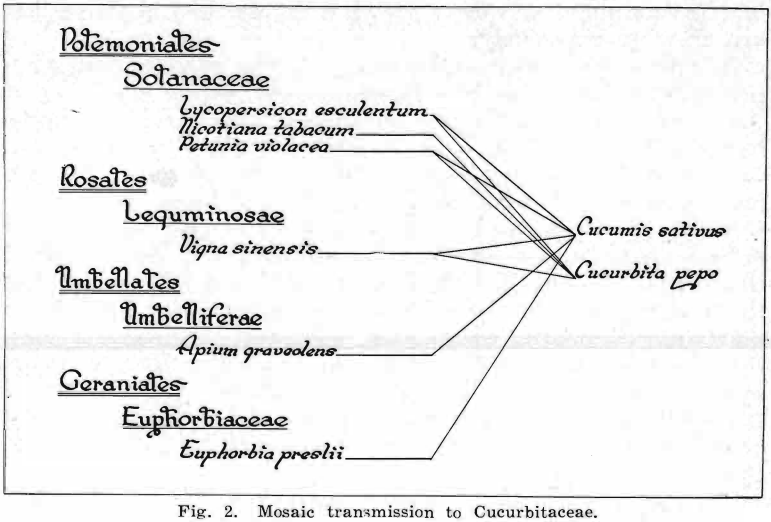

Fig. 2. Mosaic transmission to Cucurbitaceae.

infection occurred, however, the symptoms were similar to those resulting from inoculations with mosaic virus from tobacco or tomato.

Of importance in comparing pathogenic agencies that affect a number of species, is their comparison on some common host. When this comparison is made to secure data concerning the specificity of these agencies the symptoms produced on the common host offer a better index than will a comparison of symptoms on the different hosts.

Tobacco and tomato were used in these investigations as common hosts on which mosaic symptoms were compared following infection from species belonging to different families and orders. The adaptability of tobacco for use as a common mosaic host is especially fortunate as the mosaic disease was

first described on this plant and this disease has, in addition, been most extensively investigated on tobacco. The symptoms of mosaic exhibited on tobacco may be considered the type symptoms for this disease.

A very important factor contributing to success in the cross infection of mosaic among plants belonging to a wide host range was the utilization of vigorously growing plants for the inoculations. Environmental conditions such as heat, light, moisture and food supply are the factors that regulate this vigor of growth. These factors are of importance in influencing the reaction of plants infected with the mosaic virus, both as concerns the length of the incubation period and the character of the symptoms. The mosaic incubation period is longer in stunted or slowly growing plants than in those that are growing vigorously.

In numerous instances the length of the mosaic incubation period was dependent to a larger degree on the condition of the inoculated plant than on the source from which the inoculum was taken. Thus tobacco plants inoculated with the mosaic virus from celery became infected in 14 days; tobacco plants inoculated with mosaic cucumber tissue became infected in 12 days; and cucumber plants inoculated with the mosaic virus from celery became infected in 9 days. In all these cases the length of the incubation period was well within the time required for infection to become apparent when these plants are inoculated with mosaic virus from the same species. Because the length of the incubation period is largely determined by the growth vigor of the inoculated plants, it would seem that differences in the length of the incubation period may be rather an index of growth vigor than an indication that different viruses are present.

Having shown that the mosaic causal agent is transmissible over a wide host range, the question naturally arises how such transmission is effected in the open. It has already been clearly shown, not only in this paper, but by Allard (1), Doolittle (24), Brandes (10) and others, that insects are very efficient vectors. Mosaic infections among species belonging to different families obtained in these investigations thru the agency of insects are shown in table XV. These results indicate that insects are important not only because they effect a general spread of this disease within a given species, but also because they are able to transmit mosaic infection among different families and orders.

A list of plants that are known to be susceptible to mosaic and that are hosts for seven species of insect mosaic vectors, is presented in table XVII. Wilson and Vickery (63) report *Aphis gossyppi* Glover as attacking 54 species of plants; *Aphis maidis*

TABLE XVII. REPORTED HOST RANGE OF CERTAIN INSECT VECTORS OF THE MOSAIC VIRUS

Aphis gossypii (63)	*Aphis maidis* (63)	*Rhopolosiphum persicae* (63)
Beta vulgaris	*Digitaria sanguinales*	*Beta vulgaris*
Cucumis melo	*Echinochloa colona*	*Citrullus vulgaris*
Cucumis sativus	*Eleusine indica*	*Cucumis melo*
Cucurbita maxima	*Panicum sp.*	*Lycopersicon esculentum*
Cucurbita pepo	*Saccharum officinarum*	*Nicotiana rustica*
Datura stramonium	*Setaria sp.*	*Nicotiana tabacum*
Lycopersicon esculentum	*Sorghum saccharatum*	*Solanum dulcamara*
Phaseolus lunatus	*Zea mays*	*Solanum melongena*
Phaseolus vulgaris	*Achyrodes aureum**	*Solanum nigrum*
Saccharum officinarum		*Solanum tuberosum*
Solanum tuberosum		*Trifolium pratense*
Trifolium pratense		*Trifolium repens*
Vicia faba		*Zea mays*

Macrosiphum selanifolii (63)	*Pseudoccus maritimus**	*Diabrotica vittata* (16)	*Diabrotica duodecim-punctata* (15)
Amaranthus retroflexus	*Apium graveolens*	Cucurbitaceae	Beets
Brassica rapa	*Cucumis sativus*	Beans	Cucumber
Phaeolus vulgaris	*Cucumis melo*	Peas	Cantaloupe
Physalis sp.	*Cucurbita pepo*	Sunflower	Pumpkin
Pisum sativum	*Calendula officinalis*	Corn	Squash
Solanum melongena	*Heliopsis scabra*	Sugar beets (51)	Watermelon
Solanum tuberosum	*Lycopersicon esculentum*		Milkweed
	Nepeta cataria		Cabbage
	Nicotiana alata		Turnip
	Nicotiana quadrivalus		Canna
	Nicotiana tabacum		Sweet Pea
	Petunia violacea		Tomato
	Phaseolus vulgaris		Tobacco
	Solanum tuberosum		Pokeweed
	Solanum melongena		Raspberry
	Saccharum officinarum		Potato
	Soja max		Horse nettle
	Vigna sinensis		Crimson clover
	Zea mays		Sunflower
	Zinnia elegans		Grasses (32)
			Egg plant (32)
			Red clover (32)
			Soybean (61)
			Cowpea (61)
			Corn leaves (60)
			Alfalfa (32)
			Physalis*

*Observed by the writer.

Fitch, 17 species; *Rhopalosiphum persicae* Sulzer, 176 species; and *Macrosiphum solanifolii* Ashmead 19 species. The range of these four species of aphids extends to plants that are widely separated taxonomically, including species in the Monocotyledoneae and the Dicotyledoneae. An additional host was found for *Aphis gossypii**, namely, celery *(Apium graveolens)*. Celery plants growing in the greenhouse were found heavily infested with this species and the infestation continued more than three months.

Mealy bugs *(Pseudococcus maritimus* Ehr.) were found attacking many species of plants in the greenhouse of which the 20 species listed in table XVII are susceptible to mosaic.

Not only are sucking insects known to utilize different species of plants as hosts, but leaf-eating insects are likewise known to

*Determined by Dr. E. M. Patch, Maine Agr. Exp. Sta.

be cosmopolitan in their feeding habits. *Diabrotica vittata,* the striped cucumber beetle and *Diabrotica duodecimpunctata,* the twelve-spotted cucumber beetle, which is the adult of the southern corn root worm, are probably the most important of the leaf eating beetles that transmit mosaic. Both species occur generally thruout the United States from the Atlantic seacoast to the Rocky Mountains and from Canada south into Mexico (Chittenden, 17 and 15). These insects are very active, especially on warm days. They have been observed in great numbers flying from plant to plant, and unlike aphids, which often remain on a single plant for a number of generations, may feed on a number of species the same day. The twelve-spotted cucumber beetle was observed feeding on mosaic Physalis plants in a cucumber field. Walker (59) found that mosaic is transmissible between cucumber and Physalis and primary mosaic infection of cucumbers may thus be brought about by the beetles.

The striped cucumber beetle *(Diabrotica vittata* Fab.) *is* known to feed on a number of species of plants that are subject to mosaic infection as indicated in table XVII.

The twelve-spotted cucumber beetle *(Diabrotica duodecimpunctata* Oliv.) includes a much wider list of plant species in its host range. Webster (61) says that "* * * a list of its food plants would be more interesting for what it did not include * * *." In table XVII are included mosaic susceptible species that are attacked by this beetle.

The cosmopolitan feeding habits of insect vectors of the mosaic virus, together with the fact that transmissibility of this virus extends among species that are widely separated taxonomically, suggest clearly the importance of insects as agents for mosaic transmission among plants belonging to different families or orders.

SUMMARY

1. The mosaic virus is transmissible among species belonging to different families and orders. Fifteen inter-family and 11 inter-order transmissions were obtained.

2. Successful infection was obtained to tobacco *(Nicotiana tabacum)* from mosaic plants belonging to the following species: sugar cane *(Saccharum officinarum),* corn *(Zea mays),* bean *(Phaseolus vulgaris),* raspberry *(Rubus strigosus),* cucumber *(Cucumis sativus),* crookneck squash *(Cucurbita pepo* var. *condensa),* zinnia *(Zinnia elegans),* calendula *(Calendula officinalis), Stokesia laevis,* celery *(Apium graveolens),* velvet leaf *(Abutilon theophrasti),* milkweed *(Asclepias syriaca),* columbine *(Aquilegia coerulea* and *A. canadensis),* spurge *(Euphorbia preslii)* and martynia *(Martynia louisiana).*

Tomato *(Lycopersicon esculentum)* was infected with mosaic virus from sugar cane, bean, crookneck squash, zinnia, calendula, celery, velvet leaf, milkweed, catnip *(Nepeta cataria)* and martynia.

The mosaic virus was transmitted to cow pea from egg plant *(Solanum melongena)*, potato *(S. tuberosum)*, cucumber and crookneck squash.

Petunia *(Petunia violacea)* became infected with the mosaic virus from cucumber and crookneck squash; the virus from infected celery and spurge plants was transmitted to cucumbers; *Nicotiana alata* became infected with the virus from bean.

3. Ten new hosts of mosaic were found. Cross inoculations proved the disease in these to be infectious to plants belonging to the same species, to tobacco, or to tomato. The ten species are: *Achyrodes aureum, Aquilegia coerulea, Aquilegia canadensis, Euphrorbia preslii, Abutilon theophrasti, Nepeta cataria, Zinnia elegans, Calendula officinalis, Heliopsis scabra* and *Stokesia laevis.*

4. Mosaic virus transmitted from sugar cane, bean, celery and other hosts to tobacco and tomato produced mosaic symptoms similar to the symptoms occurring on tobacco or tomato infected with the virus from tobacco.

5. Vigorously growing plants, following infection, were found to have a shorter mosaic incubation period than slowly growing plants.

It appears that the masking of mosaic symptoms is not coordinate with attenuation of virulence of the mosaic virus.

6. Mealy bug *(Pseudococcus maritimus* Ehr.) and the tobacco horn worm *(Protoparce sexta* Johan.) appear to serve as agents for mosaic transmission. The tobacco horn worm transmitted the mosaic virus from infected to healthy tobacco.

7. Insect vectors of the mosaic virus served as agents for mosaic transmission among plants belonging to different families and orders. Mosaic cross infections were effected by aphids from potato and cucumber to cow pea and from celery to cucumber. Mealy bugs as vectors transmitted mosaic virus from crookneck squash to tomato and tobacco. They also transmitted mosaic virus from infected crookneck squash and egg plant to cow pea.

8. Quantitative comparative determinations of the four unit pigments of chlorophyll in healthy and mosaic leaves showed that the dark green areas of mosaic tobacco leaves contain more phytochlorin and phytorhodin than healthy leaves; that the light green areas contain less phytochlorin and phytorhodin than healthy leaves. Xanthophyll was present in less, and carotin was in abnormally large amounts in both the dark and light green areas of mosaic tobacco leaves.

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